

LLOYDIA

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The Alkaloids and Taxonomy of *Veratrum* and Related Genera¹

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Plants of the *Veratrum* group have been used for medicinal purposes for hundreds of years. Early use in the Middle Ages for sorcery and mystical rites was followed by prescription in the treatment of fevers, as local counter-irritants in neuralgia, as cardiac tonics, as emetics, as crow poisons and as insecticides (1,2). The use of *Veratrum* in the control of hypertension is at least one hundred years old (3). Early results achieved with the plant drug and with crude alkaloid extracts were erratic. Subsequent careful pharmacological investigation of purified alkaloid preparations demonstrated that the alkaloids were suitable for clinical trials. These clinical trials were followed by introduction of veratrum alkaloid preparations into clinical practice in the treatment of certain types of hypertension (4-6).

The alkaloids which have received most attention have been obtained from various species of the genera *Veratrum*, *Schoenocaulon*, and *Zygadenus*. In practice, the compounds isolated from several genera related to *Veratrum* have been classified as "Veratrum alkaloids", and it has been proposed that the latter term be defined as embracing those alkaloids isolated from plants which belong to the tribe *Veratreae* (7). The present paper surveys the occurrence and known structures of alkaloids isolated from the *Veratreae*, the classical botanical taxonomy of the *Veratreae*, and the implications of alkaloid occurrence and structure to the taxonomy of the *Veratreae*.

OCCURRENCE AND STRUCTURES OF THE VERATRUM ALKALOIDS²

In table 1 the literature on the occurrence of the veratrum alkaloids is summarized. Alkalamines of known structure are listed first, in order of increasing complexity. The three glycosidic alkaloids follow. The ester alkaloids are listed next; the latter are classified in the order of increasing complexity of the parent alkalamines. There follow a group of other compounds of known structure and, finally, a group of miscellaneous alkaloids of unknown or partially-elucidated structure. Footnotes to table 1 include the botanical source cited in the references, the geographic origin given, and the supplier where cited. Because of ambiguities in the nomenclature, the geographic location is considered important as a check on identification (see section on taxonomy below).

¹This is Part XLVIII of a series entitled "Veratrum Alkaloids"; Part XLVII, S. M. Kupchan, J. Pharm. Sci., accepted for publication.

²S. M. K. and A. A.

Alkaloids are probably present in all parts of *V. album* and *V. viride* (19,27), but the insecticidal action of dried *S. officinale*, characteristic of cevadine and veratridine, is found only in the seeds (77). The usual sources are the roots and rhizomes of *V. album* and *V. viride* and the seeds of *S. officinale*. The alkaloids

TABLE 1. Occurrence of *Veratrum* alkaloids.

Alkaloid	Formula	Sources	References
Alkamines of known structure			
Veratramine (I).....	$C_{27}H_{39}O_2N$	a,b,c,d,e,f,g,h,i	8-16,40
Rubijervine (II).....	$C_{27}H_{43}O_2N$	a,c,e,i,j,k,l,m,n,o, p,q,r,s,t,u	8,10,12,15-28,40
Isorubijervine (III).....	$C_{27}H_{43}O_2N$	a,c,e,j,k,s,v	8,10,12,16,17,25,29,40
Jervine (IV).....	$C_{27}H_{39}O_3N$	a,b,c,d,e,f,g,h,i,j,k, l,m,n,o,p,q,r,s,t,w, x,y,jj	8-10,12-24,26,40,30-34,60
Zygadenine (V).....	$C_{27}H_{43}O_7N$	z,aa	35,36
Veracevine (VI).....	$C_{27}H_{43}O_8N$	bb,cc,dd	37-39
("protocevine")			
Cevine (VII).....	$C_{27}H_{43}O_8N$	dd	38
Cevagenine (VIII).....	$C_{27}H_{43}O_8N$	dd	38
Germinine (IX).....	$C_{27}H_{43}O_8N$	c,e,r,aa	10,12,24,36
Protoverine (X).....	$C_{27}H_{43}O_9N$	ee	
Glycosidic alkaloids			
Veratrosine (XI).....	$C_{33}H_{49}O_7N$	c,ff	8,40,41
Isorubijervosine (XII).....	$C_{33}H_{53}O_7N$	ff	8,40
Pseudojervine (XIII).....	$C_{33}H_{49}O_8N$	a,c,m,o,p,q,r,s,w,ff	8,19,21-24,30,33,40,41
Ester alkaloids			
a) Esters of zygadenine			
Zygacine (XIV).....	$C_{29}H_{45}O_8N$	h,u,gg,hh,ii	14,28,42-44
Angeloylzygadenine (XV).....	$C_{32}H_{49}O_8N$	g	13,45
Vanilloylzygadenine (XVI).....	$C_{35}H_{49}O_{10}N$	aa,hh	36,43
Veratroylzygadenine (XVII).....	$C_{36}H_{51}O_{10}N$	f,l,n,u,w,aa,ff,hh	13,28,30,36,43,46,47
b) Esters of veracevine			
Cevacine (XVIII).....	$C_{29}H_{45}O_9N$	bb,cc	37,39
Cevadine (XIX).....	$C_{32}H_{49}O_9N$	bb,cc,dd,kk,ll	37-39,48-53
Vanilloylveracevine (XX).....	$C_{35}H_{49}O_{11}N$	kk	54
("vanilloylcevine")			
Veratridine (XXI).....	$C_{36}H_{51}O_{11}N$	bb,cc,dd,kk,ll	37-39,48,51,53
c) Esters of germinine			
Germitetrine (XXII).....	$C_{41}H_{63}O_{14}N$	n,mm,nn	55-59
("germitetrine-B")			
Germitrine (XXIII).....	$C_{39}H_{61}O_{12}N$	e,j,k,n,oo,pp	12,16,17,29,61,62
Neogermitrine (XXIV).....	$C_{36}H_{55}O_{11}N$	a,j,n,w,ff,gg,hh, oo,pp	8,16,30,40,43,46,56,61-64
Germanitrine (XXV).....	$C_{39}H_{59}O_{11}N$	w	30
Germinitrine (XXVI).....	$C_{36}H_{57}O_{11}N$	w	30
Germerine (XXVII).....	$C_{37}H_{59}O_{11}N$	j,l,m,n,r,t,jj,nn,pp	16,18,19,26,59,62,65,66
Germidine (XXVIII).....	$C_{34}H_{53}O_{10}N$	e,j,k,gg,oo	12,16,17,61,63,64
Neogermidine (XXIX).....	$C_{34}H_{53}O_{10}N$	c,r,gg,hh	43,62-65
("Isogermidine")			
Germbudine (XXX).....	$C_{37}H_{59}O_{12}N$	c,j,r	16,62,65
Neogermbudine (XXXI).....	$C_{37}H_{59}O_{12}N$	c,j,mm	16,58,62
Protoveratridine (XXXII).....	$C_{32}H_{51}O_9N$	o,r,gg	21,24,63,64
d) Esters of protoverine			
Protoveratrine.....		c,j,l,m,n,o,r,t,v, mm,nn,pp,qq	16,18-21,26,27,29,55,57- 59,62,65,67-69
[including			
protoveratrine A (XXXIII)	$C_{41}H_{63}O_{14}N$		
("veratrine") and			
protoveratrine B (XXXIV)	$C_{41}H_{63}O_{15}N$		
("neoprotoveratrine")]			
Escholerine (XXXV).....	$C_{41}H_{61}O_{13}N$	a,ff	8,40,46

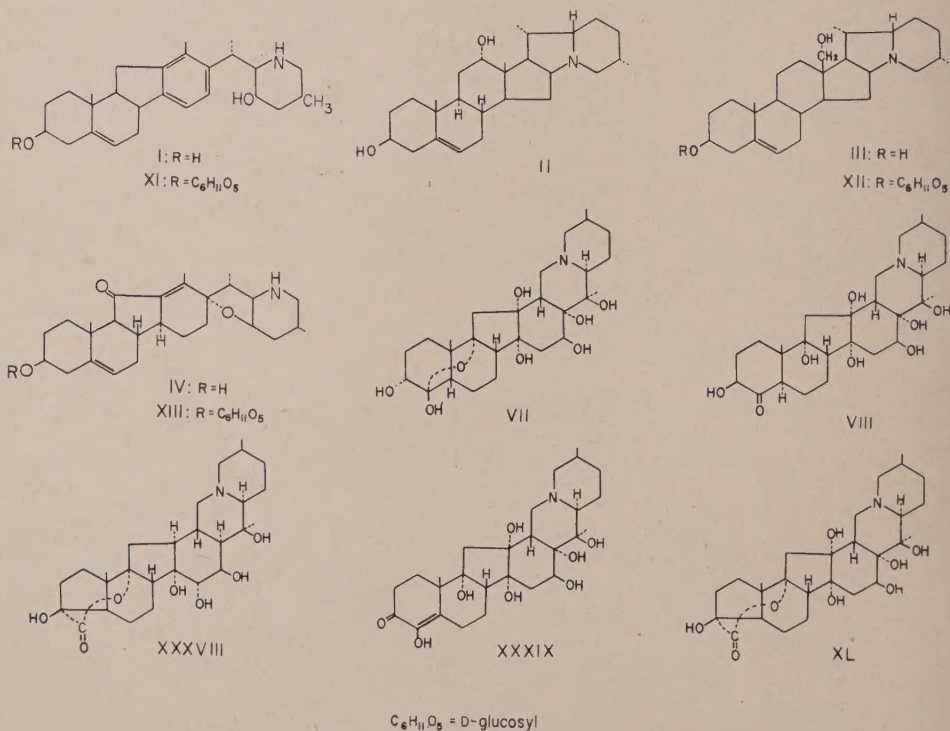
TABLE 1. *Continued.*

Alkaloid	Formula	Sources	References
Desacetylprotoveratrine A (XXXVI).....	$C_{39}H_{61}O_{13}N$	mm	58
Desacetylprotoveratrine B (XXXVII).....	$C_{39}H_{61}O_{14}N$	c,r,mm	58,62,76
Other alkaloids of known structure			
Zygadenilic acid δ -lactone (XXXVIII).....	$C_{27}H_{41}O_7N$	rr	70,71
Dehydrocevageneine (XXXIX).....	$C_{27}H_{41}O_8N$	dd	38
Cevinilic acid δ -lactone (XL).....	$C_{27}H_{41}O_8N$	dd	38
Angeloyl ester of zygadenilic acid δ -lactone (XLI).....	$C_{32}H_{47}O_8N$	i	15
Miscellaneous alkaloids			
Geralbine (XLII).....	$C_{22}H_{33}O_2N$	n	67
Synaine (XLIII).....	$C_{24}H_{39}ON$	ss	72,78
Veratrobazine (XLIV).....	$C_{24}H_{37}O_3N$	n	67
Verine (XLV).....	$C_{25}H_{39}O_2N$	ss	72,78
Rubiverine (XLVI).....	$C_{25}H_{39}O_2N$	ss	72,78
Amianthine (XLVII).....	$C_{27}H_{41}O_2N$	x	31
Isojervine (XLVIII).....	$C_{27}H_{39}O_3N$	c	41
Unnamed alkamine (Kupchan's) (XLIX).....	$C_{27}H_{43}O_3N$	j	16
Unnamed alkamine (Jacobs') (L).....	$C_{27}H_{41}O_4N$	c	41
Unnamed alkamine (Fried's) (LI).....	$C_{27}H_{41}O_5N$	k	17
Sabine (LII).....	$C_{27}H_{45}O_7N$	dd	73
("neosabadine")			
Hydroalkamine-S (LIII).....	$C_{27}H_{45}O_8N$	dd	38
Veratralbine (LIV).....	$C_{28}H_{43}O_5N$	p,q	22,23
Sabadine (LV).....	$C_{29}H_{47}O_8N$	dd,kk	73-75
("sabatine")			
Veragenine (LVI).....	$C_{31}H_{53}O_{13}N$	cc	39
Verabidine (LVII).....	$C_{37}H_{61}O_{12}N$	n	20,67

(a) *Veratrum eschscholtzii* Gray; Alaska. (b) *V. stamineum* Maxim; Japan. (c) *V. viride* Ait; S. B. Penick and Co. (d) *V. grandiflorum* Loes. fil.; Noppo, Japan. (e) Verabore, a commercial prep. from *V. viride*; S. B. Penick and Co. (f) *V. album* var. *oxysepalum*; Hokkaido, Japan. (g) *V. album stamineum* Maxim; Nagano, Japan; Aug., 1956. (h) *V. grandiflorum* Loesen.; Nagano, Japan; 1956. (i) *V. grandiflorum* Loesen.; Hokkaido, Japan; June, 1957. (j) Cryptenamine, a commercial prep. from *V. viride*; Irwin, Neisler and Co. (k) *V. viride*; N. Carolina. (l) *V. viride*; E. Merck, Darmstadt, imported from USA. (m) *V. album* Caesar and Loretz (Suppliers). (n) *V. album*. (o) *V. viride*; Gehe and Co., Dresden. (p) *V. album*; Hopkin and Williams (Suppliers). (q) *V. viride*; Hopkin and Williams. (r) *V. viride* Ait. (s) *V. album* var. *lobelianum*; Eastern Slovakia on "Čerhovské pohorí". (t) *V. album*; Yugoslavia. (u) *V. oxysepalum* Turcz.; Hokkaido, Japan; Aug., 1956. (v) *V. viride* Ait.; Quebec; Summer, 1950. (w) *V. fimbriatum* Gray; Northern California; Summer, 1950. (x) *Amianthium muscaetoxicum* Gray. (y) *V. lobelianum*; Poland. (z) *Zygadenus intermedius*; Wyoming. (aa) *Z. venenosus* Wats.; Washington; June, 1950. (bb) Veratrine, a commercial prep. from *Schoenocaulon officinale* S. B. Penick and Co. (cc) Veratrine sulfate, a commercial prep. from *S. officinale*; E. Merck, Darmstadt; Lot No. 54618-4080. (dd) Veratrine, a commercial prep. from *S. officinale*; E. Merck, Darmstadt. (ee) Isolation of the alkamine protoverine has apparently not been reported; inclusion here is based on wide occurrence of protoverine esters in *V. spp.* (ff) *V. eschscholtzii* Gray; Alaska; Summer, 1950. (gg) *Z. venenosus*; northeastern Oregon; June, 1951. (hh) *Z. paniculatus*; Washington; June, 1951. (ii) *V. album* var. *grandiflorum* Loes. fil. (jj) *V. nigrum*; Botanical gardens, Univ. Wurzburg. (kk) *S. officinale*; S. B. Penick and Co. (ll) Veratrine; commercial. (mm) *V. album*; S. B. Penick and Co. (nn) Protoveratrine, commercial. (oo) *V. viride*; Eastern USA; Summer, 1948 and 1949. (pp) *V. viride*; S. B. Penick and Co.; 1952. (qq) *V. album*; Poland. (rr) *V. album* var. *oxysepalum*; Hokkaido, Japan; Aug., 1956. (ss) *V. album*, Sinaia, Roumania; 1953.

can be extracted from the appropriate parts of the dried and powdered plants by aqueous or alcoholic acid or by organic solvents, usually with added base in the form of ammonia or triethylamine. Subsequent separation of the bases from the crude extract has been achieved by fractional crystallization, precipitation or extraction (21-23,41), and by chromatographic separations on alumina (37,56), on silica gel (49,75), on kieselguhr (27), and on Celite (16). Chromatography on paper has proved invaluable for characterizing the alkaloids (16,27,55). Perhaps the most useful technique for the isolation of the individual ester alkaloids from amorphous alkaloid mixtures has been liquid-liquid counter-current distribution (e.g., 17,29,55,62).

FIGURE I
ALKALOIDS OF KNOWN STRUCTURE

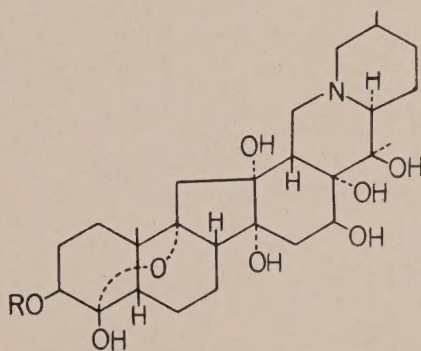
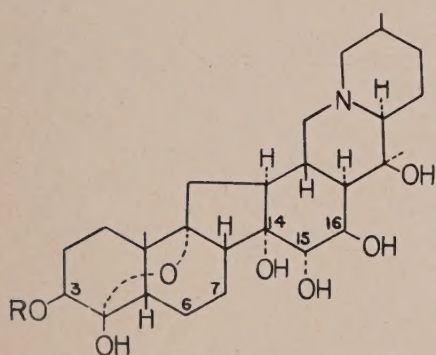


The elucidation of the structures of the veratrum alkalamines has been summarized in several recent comprehensive reviews (79-81), and the work on the ester alkaloids has also been surveyed in a recently-completed review (82). Consequently, no detailed account of the structure elucidation is undertaken here. It does appear appropriate, however, to make a few comments concerning the classification and interrelationship of the members of the series.

Figures 1-3 present the constitutions of those alkaloids for which complete structures have been elucidated. Thus far, only the C₂₇ alkalamines and their derivatives have received the chemical study necessary for complete structure elucidation. The C₂₇ alkalamines fall into two distinct chemical groups: the jerveratrum group, which includes veratramine (I), rubijervine (II), isorubijervine (III), and jervine (IV), and the ceveratrum group, which includes zygaenine (V), veracevine (VI); germinine (IX), and protoverine (X) (79). The jerveratrum

unconjugated alkaloids contain only two or three atoms of oxygen and are found in unhydrolyzed plant extracts in part as the free alkamines and in part in combination with one molecule of D-glucose as glyco-alkaloids (e.g., XI, XII, XIII). The ceveratrum bases are highly hydroxylic and contain seven to nine atoms of oxygen; they usually occur esterified with various acids as ester alkaloids; they have never been found as glycosides. Isolation of the free ceveratrum alkamines has been reported from many laboratories. However, the fact that the isolation procedures generally involved the use of alkaline conditions which may have led to hydrolysis has left unanswered the question as to the occurrence of free ceveratrum alkaloids *in the plant*.

FIGURE 2
ALKALOIDS OF KNOWN STRUCTURE



V : R = H
XIV : Ac
XV : An
XVI : Va
XVII : Ve

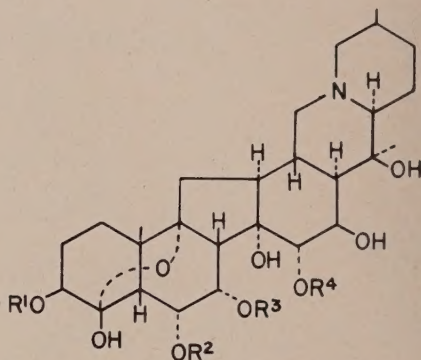
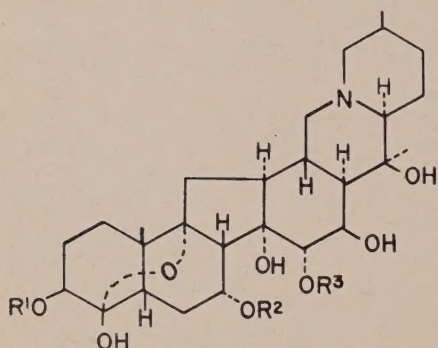
VI : R = H
XVIII : Ac
XIX : An
XX : Va
XXI : Ve

Ac = acetyl; An = angeloyl; Va = vanilloyl;
Ve = veratroyl.

The jerveratrum alkamines rubijervine (II) and isorubijervine (III) may be regarded as the simplest of the veratrum alkaloids from a structural point of view. The latter compounds each have the normal C_{27} -steroid skeleton (e.g., cholesterol), and the E and F rings may formally be regarded as having been formed by folding the normal cholesterol side chain around the nitrogen atom. The positions of oxygen-bearing carbon atoms 3, 12 and 18 are the same as those of several non-nitrogenous naturally-occurring steroids. Veratramine (I) and jervine (IV) are characterized by the C-nor-D-homo ring system, which may formally be regarded as having originated by migration of the C_{13}, C_{14} -bond of a normal steroid to the C_{12}, C_{14} -position. As noted above, three of the four jerveratrum alkamines also occur as glycoalkaloids, conjugated with one molecule of glucose.

The four highly hydroxylated native ceveratrum alkalamines are veracevine, germine, protoverine and zygaenine. Cevagenine and cevine are known to result from base-catalyzed isomerization of veracevine, and the isolation of the latter alkaloids from veratrine (a commercial alkaloid extract of *Schoenocaulon officinale*) is probably attributable to isomerization during the extraction and isolation procedure. Similarly, dehydrocevagenine is probably formed by auto-

FIGURE 3
ALKALOIDS OF KNOWN STRUCTURE



	R ¹	R ²	R ³
IX :	H	H	H
XXII :	HMAB	Ac	MB
XXIII :	MB	Ac	HMB
XXIV :	Ac	Ac	MB
XXVII :	An	Ac	MB
XXVIII :	Ac	H	MB
XXIX :	H	Ac	MB
XXX :	t-DMB	H	MB
XXXI :	e-DMB	H	MB

	R ¹	R ²	R ³	R ⁴
X :	H	H	H	H
XXXIII :	HMB	Ac	Ac	MB
XXXIV :	t-DMB	Ac	Ac	MB
XXXV :	An	Ac	Ac	MB
XXXVI :	HMB	Ac	H	MB
XXXVII :	t-DMB	Ac	H	MB

Ac = acetyl; An = angeloyl; e-DMB = (l)-erythro-2,3-dihydroxy-2-methylbutyryl; t-DMB = (d)-threo-2,3-dihydroxy-2-methylbutyryl; HMB = (d)-2-hydroxy-2-methylbutyryl; HMAB = erythro-2-hydroxy-2-methyl-3-acetoxybutyryl; MB = (l)-2-methylbutyryl.

oxidation during the extraction and isolation process [cf. (83)]. The four native ceveratrum alkalamines have several common structural features. All four possess the modified steroid cevan nucleus. The latter skeletal structure is characterized by the aforementioned C-nor-D-homo arrangement along with an alternate folding of the normal cholesterol side chain around the nitrogen atom. Another characteristic feature of the four native ceveratrum alkalamines is the α -ketol hemiketal

system found in rings A and B. Zygadenine, germine and protoverine have identical structures in rings C,D,E and F. Protoverine has been degraded to a germine derivative (84) and germine to a zygadenine derivative (85) by suitable alteration of ring B. Veracevine differs appreciably from the other three in the distribution of functional groups on rings C,D,E and F of cevan skeleton. On the other hand, veracevine and zygadenine have identical structures in rings A and B, and this resemblance may figure in the similar pattern of esterification in the naturally-occurring ester derivatives of the latter alkalamines. The similarity of the rings A and B structures of veracevine and zygadenine may account also for the occurrence of analogous lactone derivatives. Cevinilic acid δ -lactone (XL) [first prepared by chemical oxidation (86)] and the analogous zygadenilic acid δ -lactone (XXXVIII) have been found, but no analogous derivatives of germine or protoverine have been described to date.

The ester alkaloid derivatives of the ceveratrum alkalamines fall into two groups. All the zygadenine and veracevine esters isolated to date are monoesters with either acetyl, angeloyl, veratroyl or vanilloyl residues affixed at C₃. All the naturally-occurring germine and protoverine esters, on the other hand, are polyesters, with tri- and tetra-esters predominant. (Protoveratridine, a minor germine monoester, is probably to be regarded as an artifact which arises by alkaline hydrolysis of polyesters during the isolation procedure.)

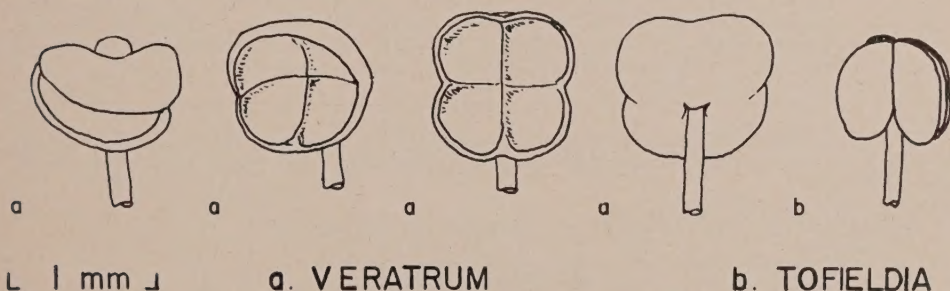


FIG. 4. Diagrams illustrating the type of anther found (a) in the tribe Veratreae and (b) in genera related to, but excluded from, the Veratreae (such as *Chamaelirium*, *Chionographis*, *Tofieldia*, *Helonias*). In *Chionographis*, the two anther cells are sometimes confluent (106).

From the chemical point of view, then, zygadenine represents something of a hybrid structure among the ceveratrum alkaloids. On the one hand, zygadenine occurs alongside germine and protoverine in a number of plants, and possesses a ring C,D,E and F structure identical with those of germine and protoverine. On the other hand, zygadenine possesses a ring A,B structure identical with that of veracevine, is a formal precursor for a naturally-occurring δ -lactone derivative analogous to one formally derived from veracevine, and occurs in monoester conjugates which are closely analogous to the monoester conjugates of veracevine.

CLASSICAL BOTANICAL TAXONOMY OF THE VERATREAE³

The tribe Veratreae is part of the subfamily Melanthioideae of the family Liliaceae (87-90). Sometimes this subfamily is treated as a separate family, the Melanthiaceae (91,92), or the Colchicaceae (93). While some of the other genera in this subfamily share the separate styles and septicidal capsules found throughout the tribe Veratreae, this tribe possesses a unique type of anther (88,94). Although allied but excluded genera possibly exhibit a transitional anther structure (fig. 4), the tribe is sufficiently well delimited by its unusual anther that only rarely (92,95) are its genera apportioned among different tribes.

³J. H. Z.

Four generic names in common usage provide a convenient subdivision of the tribe into four major groups of species (table 2): *Veratrum* (false hellebore), *Zygadenus* (death camas), *Stenanthium*, and *Schoenocaulon* (Sabadilla). To the vernacular names chosen here (96), may be added many others (89,92,95,99,100, 113,116,118,154). Two other well-known genera, *Amianthium* (crow poison) and *Melanthium* (bunch flower), are here placed under *Zygadenus* and *Veratrum*, respectively, for reasons given below.

TABLE 2. Botanical definition of the species of the tribe Veratreae

Information given includes: Name used in this paper^a; geographic range and habitat; distinguishing morphological features (those unique in the tribe are starred^{*}; legend for abbreviations given below^b); and names used in selected references (parentheses denote "in part").

VERATRUM. N Hemisphere. Pub* (exc 1); winged seeds* (exc 1); bulb and short to long rhizome.

ALBOVERATRUM*. Circumboreal; mt and tundra (exc Himalayas and N and C Canada). T erose to toothed, usu ascending in fruit; gland V-shaped*; lvs broad (elliptic), usu pub beneath; vegetative pseudoculms tall*; styles central; 2° rac usu compound; ped usu very short; lower fls usu staminate; rhizome stout. Section *Alboveratrum* 94,95; Subgenus *Euveratrum*, Sect. *Alboveratrum* (97,98).

V. album. Eurasia; W Alaska. Variable; by elimination of other species.

album. N Portugal E to Poland and Greece; NW Turkey; Caucasus. Mt. meadows. Highly variable in all features; t generally intermediate in size and shape, green to yellow or white. *V. album* L. 93,98-101, 123,(88), ssp. *album* 102,103, ssp. *lobelianum* (Bernh.) Hult. (102,103); *V. lobelianum* Bernh. (88,93,98); *V. bosniacum* Beck 98; *V. croaticum* (Beck) Loes. 98; *V. fluviu* (Griseb.) Loes. 88,98.

^aA few unpublished names or name combinations (such as Section *Eustenanthium*) are used here for convenience in summarizing information only. All names are ranked in the table as follows:

GENUS (In the broad sense)

SUBGENERIC GROUP (Section; or Genus in the narrow sense)

Species

Intraspecific unit (Subspecies or variety)

^bLegend for abbreviations: N, north, northward, etc.; C, central; mt, mountain(s); *infl*, inflorescence; *rac*, raceme(s); 2° *rac*, secondary (branch) raceme(s); t, tepals; *ped*, pedicel(s); *lvs*, leaves; *fl*, flowers or flowering; *pub*, pubescent; *sub*, almost, mod, moderately; *usu*, usually; *rel*, relatively; *esp*, especially; *exc*, except; *lat*, latitude; *alt*, altitude.

^cSection *Alboveratrum* is interpreted as follows: *V. stamineum*, *V. insolitum*, and *V. dahuricum* rate full specific rank by virtue of constancy of their unique features despite sympatry with other *Alboveratra*. All seven American *Alboveratra* rate specific rank because of the relative stability of their distinguishing features or combinations of them. The Eurasian *V. album*, in contrast, forms a complex of broad geographic clines to which there are many striking local exceptions in all regions [such as long ped and internodes and subglabrous lvs (95, "V. patulum") in S Japan; local variability in Kamtchatka (102)]. The Asian cline is arbitrarily separated into two subspecies (*grandiflorum* to the south; *oxysepalum* to the north) in the zone where intermediate and heterogeneous populations prevail, in Manchuria, N Korea, Ussuri Region of SE Siberia, Far East (Maritime Terr.), Sakhalin, and Hokkaido (89,95,104,107,108).

The populations in Arctic Europe are placed with ssp. *oxysepalum* because of weak morphological tendencies and Hulten's interpretation (103,113) of the history of *V. album*. The remaining heterogeneous assortment of populations is grouped under ssp. *album* in the table. The green flowered forms, often called *V. lobelianum* (or *V. album* var. *lobelianum*), include a great diversity of forms and reach all the geographic boundaries given for ssp. *album*. Typical *V. album* (large, broad white tepals) is less widely distributed; it prevails in Hungary, E Austria and parts of Yugoslavia, and grades into smaller-flowered and more variable forms toward W Austria and S Germany. Sometimes green and white (and even yellow) forms are locally juxtaposed (99). Because *V. album* not only shares the phenotypic variability common to most *Alboveratra* but in addition appears to be genotypically diverse and perhaps heterozygous, it is especially essential in this species that a complete voucher specimen, with notes on exact geographic location and local ecology, be preserved for each lot of rhizomes dug, in order to insure uniformity and comparability of medical and chemical results.

The E American *V. viride*, superficially at least, appears to be an extensive, relatively stable (uniform) population similar (through history or coincidence) to a few of the thousands of diverse local populations of *V. album* in Eurasia. But in general *V. viride* has longer and more numerous branches than the green-flowered forms of *V. album*.

TABLE 2. *Continued*

- grandiflorum*. C and SW China (Yunnan, E Sikang, Szechuan, Hupeh, N Kiangsi); C and S Japan; C and S Korea; mt meadows and forests. T very large (8-18 mm long), broad, greenish white; ovary always woolly; lvs usu pub. *V. grandiflorum* (Maxim.) Loes. 98,104,105,(95); *V. album* L. 110, 116, var. *grandiflorum* Maxim. 93,106; *V. patulum* Loes. 95,98,105,107; *V. puberulum* Loes. 98; *V. sikokianum* Nakai 95; *V. dahuricum* (Turcz.) Loes. (98).
- oxysepalum*. Woods and swamps, mt of N Korea and Hokkaido, N in meadows, shores, brush and tundra to Arctic Coast from N Norway E to Nome, Alaska. T mod to small, narrow, green or yellowish or whitish green; ovary and lvs often subglabrous. *V. oxysepalum* Turcz. 89,93,95,98,104,105; *V. album* L. 108, var. *oxysepalum* (Turcz.) Miyabe and Kudo 109, var. *viride* Baker 110, ssp. *oxysepalum* (Turcz.) Hult. 102,103,111-114, ssp. *lobelianum* (Bernh.) Hult. (102,103); *V. lobelianum* Bernh. 89,115,(88,93,98), var. *asiaticum* Loes. 98,104; *V. misae* (Sirjaev.) Loes. 89,98,103; *V. dolichopetalum* Loes^d 98,107; *V. calycinum* Komarov 89; *V. alpestre* Nakai 95,107; *V. grandiflorum* Loes. (95).
- V. dahuricum*. Marshes and brushy slopes in Siberia, from Tomsk (85° E long) E to mouth of Amur R., S to mt of N Korea. Upper lvs and ovary densely white-woolly; t small, yellowish white, glabrous on the conspicuously paler margins. *V. dahuricum* (Turcz.) Loes. 88,89,107,115,(98).
- V. viride*. Wet woods, Gulf of St. Lawrence, Quebec, S to Md. and NE Ohio; S in high mt meadows to SW N. C. T rather large, often lanceolate, green; infl robust, crowded; ovary and usu lvs mod pub; fl early summer. *V. viride* Aiton 88,91-93,98,100,101,109, 117-121,123,(121).
- V. eschscholtzii*. Moist slopes, sea level to timberline, Alaska Peninsula and Mt. McKinley, Alaska, E to SE Yukon, S to higher mt of N Calif., C Idaho and SW Mont. T rather small, greenish, often oblanceolate; upper lvs finely white-woolly between veins; 2° rac strongly pendent, not crowded, with ped turning upward as soon as flowers open; ovary often mod pub; fl late summer. *V. eschscholtzii* Gray 91, 93,100,101,109,112,114,120,122,123; *V. eschscholtzianum* (Schult.) Rydb. 124,125; *V. escholtzianum* (Schult.) Loes. 88,98; *V. viride* Aiton 126-129, 133, 134, (121), var. *escholtzianoides* Loes. 98; *V. speciosum* Rydb. (125?,126); *V. californicum* Durand (91), (131?); *V. unidentified* 101?
- V. tenuipetalum*. Wet meadows, high mt of Colo. and N New Mexico. Tall; 2° rac much-branched; t small, narrow, yellowish white, translucent; upper lvs subglabrous to mod pub (esp. toward apex); ovary glabrous. *V. tenuipetalum* Heller 91,98,124, 125; *V. californicum* Durand 132, (91,93,131), var. *watsoni* Baker (93); *V. speciosum* Rydb. (126).
- V. jonesii*. Rather dry, low-elevation meadows or prairies, W Idaho or C Ore. and C Wash. T small (7.5-10.0 mm long), rel broad, creamy white; upper lvs finely white-woolly between veins; 2° rac much-branched; seeds very large (12-19 mm long); ovary glabrous. *V. jonesii* Heller 91, 98, 124; *V. speciosum* Rydb. (122,125,126); *V. californicum* Durand (127,130,131,133).
- V. caudatum*. Low elevation swamps from Cascade Mts to coast in Ore. and Wash. Sparingly branched; terminal rac very long; t large, rel very narrow, greenish white; lvs mod pub on veins to glabrous; ovary glabrous. *V. caudatum* Heller 91,98,124, 127,130,134,135; *V. californicum* Durand (52?,91?,131).
- V. californicum*. Common in low to high mt meadows from N Idaho, S Ore. and W Wyo. S to S Calif., S New Mexico, and N Mexico. T large, broad, creamy white; lvs usu mod pub on veins; ovary rarely pub; mod branched. *V. californicum* Durand 98,100,101,124,128,129,134,136-139, (91,120,125,127,130,131,133), var. *watsoni* Baker (93); *V. speciosum* Rydb. 98, (122,126); *V. caudatum* Heller var. *tenuipetaloides* Loes. 98; *V. af. californicum* 140.
- V. insolitum*. Mt slopes (serpentine and diorite of NW Calif. and W Ore. Much-branched infl and ovaries densely woolly; t white, thin, small (5-9 mm long) rel broad, often fringed; ped long, spreading; lvs pub on veins; capsule pub; seeds large. *V. insolitum* Jepson 128-130,134.
- V. stamineum*. Mt marshes, C and N Japan. T very small (about as long as stamens), rel broad, white; ped very long, spreading; infl and lvs small; ovary glabrous; lvs glabrous toward N. *V. stamineum* Maxim. 93,95,98,104,105,106,141; *V. nipponicum* Nakai (apparently a hybrid with *V. album grandiflorum*) 142.

^dThe status of these forms needs further clarification.

TABLE 2. *Continued*

- FUSCOVERATRUM*. E Asia; 1 species W to C Europe. T oblong, narrowed convexly at base, entire, reflexed in fruit; gland dark, covering most of basal $\frac{2}{3}$ to $\frac{1}{2}$ of t; anthers open early, fall soon; styles diverge from outer corners of glabrous truncate ovary; ped usu rel long, divergent; leaf blades glabrous; rhizome mod to short; 2° rac mostly unbranched. Section *Fuscoveratum* 94, (95); Subgen. *Euveratum*, Sect. *Fuscoveratum* 98, (97); Subgen. *Pseudanticlea* (97, 98); Section *Alboveratum* (97).
- V. nigrum*. Asia: Siberia from Altai down Yenisei R. to 67° N lat, E to mouth of Amur R., S to S Korea, Quelpaert I. and Sado I.; China from Jehol SW to E Sikang and W Hupeh. Europe: SW Switzerland and C Italy to SW Ukraine and Yugoslavia; Kursk. Mt slopes, forest openings, meadows. Robust; lvs elliptic; rac many, long, many-fld, usu white-woolly; t purple, usu dark. *V. nigrum* L. 88, 89, 93, 94, 98, 99, 110, (108), var. *japonicum* Baker (110), var. *ussuriense* Loes. 98, 115; var. *microcarpum* Loes. 98; *V. bracteatum* Batalin 98, 110; *V. schindleri* Loes. (98); *V. sadoense* Nakai 95; *V. ussuriense* Nakai 107.
- V. maackii*. SE Siberia and N Japan S to SW China. Variable but generally smaller, slenderer, narrower-leaved and less pubescent than *V. nigrum*.
- maackii*. Amur. R. between cities of Blagoveshchensk and Khabarovsk, S to S Korea and Shantung; low meadows, brushy slopes, woods. Lvs lanceolate; t usu purple; ped and rac usu long; capsules usu slender; in S Korea tend to be yellow-green fld and have short terminal and compound 2° racemes. *V. maackii* Regel 89, 94, 115, (93, 98, 107, 110); *V. mandshuricum* Loes. 98, 107; *V. bonhoffii* Loes. 88, 98, 107; *V. coreanum* Loes. (98, 107); *V. oblongum* Loes. (98), var. *macrantha* Loes. 98; *V. versicolor* Nakaid^d 107; *V. nigrum* L. (108).
- japonicum*. Japan: forests, meadows, mt of S Hokkaido and extreme N Honshu. Lvs elliptic; t purple; ped and rac mod length. *V. japonicum* (Baker) Loes. 94, 98, 104, 105 (95); *V. nigrum* L. var. *japonicum* Baker 93; *V. maximowiczii* Baker 104?
- maximowiczii*. Japan: forests and meadows, mt of N and C Honshu. Lvs elliptic (rarely linear); t yellow-green; ped mod length; rac often long. *V. maximowiczii* Baker 93, 94, 98, 105, 106, (95); *V. angustipetalum* Loes. (98); *V. warburgii* Loes. (98); *V. coreanum* Loes. (98).
- reymondianum*. Japan: mt forests and alpine meadows, C Honshu and Sado. Lvs elliptic to lanceolate; t purple; ped mod length; rac variable. *V. reymondianum* (Loes.) Zimmerman 94; *V. nigrum* L. var. *japonicum* Baker 106, var. *reymondianum* Loes. 97; *V. japonicum* (Baker) Loes 105 var. *reymondianum* Loes. 98, (95); *V. warburgii* Loes. (98).
- maackioides*. Japan: mt forests and meadows from C Honshu to Kyushu. Lvs narrowly lanceolate to linear; t purple; ped long; rac variable in length. *V. maackioides* Loes 94, 98, (95); *V. maackii* Regel 105 (93, 95, 107); *V. japonicum* (Baker) Loes. (143), var. *reymondianum* Loes. (95); *V. maximowiczii* Baker (95); *V. coreanum* Loes. (107).
- coreanum*. Korea: Alpine meadows, Quelpaert I. Lvs linear; t yellow-green; ped and rac mod length; 2° rac simple; plant small. *V. coreanum* Loes. 94, 107, (98); *V. maximowiczii* Baker (95).
- oblongum*. C China: moist meadows, W Hupeh, E Szechuan. Lvs elliptic, papillate on veins; t purplish, at least on gland; ped and infl long; lower rac compound; bracteoles woolly; tall plant. *V. oblongum* Loes. 94, 116, (98); *V. maackii* Regel (110); *V. maximowiczii* Baker (110).
- kiulingianum*. C China: moist woods, S Anhwei, N Kiangsi, N Kwangsi. Lvs elliptic to lanceolate, large; t yellow-green with reddish gland; ped mod length, infl long with many very short compound 2° rac; bracteoles woolly; tall plant. *V. kiulingianum* Zimmerman 94; *V. maximowiczii* Baker (110); *V. oblongum* Loes. (98); *V. warburgii* Loes. (98); *V. angustipetalum* Loes. (98); *V. cavaleriei* Loes. (98); *V. schindleri* Loes^d. 94, 116, (98).

*Section *Fuscoveratum* is interpreted as follows: The entities are all very closely related. Most of them form a network of geographically-replacing populations joined to each other by steep places in the over-all morphological gradients extending from SW China to the Amur R. and N Japan. The points of steepest clines and greatest heterogeneity are E-C China, Korea, and C Honshu. Most of the entities are here treated as subspecies of *V. maackii*. *V. nigrum* rates specific rank because it appears to maintain uniformity and distinctness [Maximowicz (108) to the contrary] where its range overlaps that of *V. maackii* from the Amur R to S Korea; moreover, it appears to be polyploid (155-157).

TABLE 2. *Continued*

- formosanum*. China: mt meadows; from NE Kweichow and N Chekiang S to Hong Kong; Taiwan; Okinawa. Often stout; lvs linear, stiff, bracteoles and purple t usu woolly; ped mod length; rac often long, even the lower ones usu fertile. *V. formosanum* Loes. 88,94,98; *V. chingianum* Zimmerman^d 94; *V. nigrum* L. var. *japonicum* Baker 110; *V. warburgii* Loes. (98); *V. kudoii* Masamune 143; *V. japonicum* (Baker) Loes. 143; *V. maackii* Regel 110?).
- atroviolaceum*. SW China: wet meadows, W Yunnan. Slender; lvs linear, flaccid; bracteoles and purple t very woolly; peds and rac mod length. *V. atroviolaceum* Loes. 94,98.
- V. longebracteatum*^d. Japan: alpine meadows, N and C Honshu. Like *V. m. maximowiczii* except: t more pointed, ascending; rac short; either bracteoles or bracts often long; sometimes subglabrous. *V. longebracteatum* Takeda 94,95,97,98,105; *V. maximowiczii* Baker (95,98).
- V. micranthum*^d. C China: E Szechuan. Lvs lance-elliptic, papillate; fls very small; plant small. *V. micranthum* Wang and Tang 144; *V. minutiflorum* Zimmerman 94.
- TELANDRIUM. Centering in the two Arcto-tertiary refugia (145), the mt of SW China and E USA. Stamens inserted on base of t*; t entire, narrowed concavely toward base; glands paired, central (exc 1); ovary usu as in *Fuscoveratrum* but sometimes pub.; leaf blades glabrous; rhizome usu poorly developed; 2° rac. sometimes branched. Sect. *Telandrium* 94; Subgen. *Pseudoanticlea* (97,98) Subgen. *Pseudomelanthium* 97, 98; Sec. *Fuscoveratrum* (95); Genus *Melanthium* 97.
- V. shanense*. SW China. T green, with small single basal gland; lvs linear to lanceolate, sometimes papillate.
- shanense*. Lower elevation thickets and wet ground, S Sikang, N Yunnan, N Burma. Lvs and rac long; bracteoles short; ped mod. length; t small, spreading. *V. shanense* W. W. Smith 88,98, var. *shanense* 94; *V. yunnanense* Loes. 88,98,146.
- stenophyllum*. High alpine meadows and forest edges, S Sikang. Rac and ped short; lvs short, blunt; bracteoles long; t large, ascend. *V. shanense* W. W. Smith var. *stenophyllum* (Diels) Zimmerman 94; *V. stenophyllum* Diels 88,98,146.
- V. anticleoides*. Far E USSR: Damp barrens and coniferous forest, Sakhalin and adjacent mainland. Small, glabrous; t greenish yellow, with soon-obscure slender purple glands; lvs linear; rhizome often long. *V. anticleoides* (Trautv. and Meyer) Takeda 94,95,98,104; *Acelidanthus anticleoides* Trautv. and Meyer 89,93,115.
- V. woodii*. E USA: Deciduous forest, S Iowa and E Okla. E to W Ohio and C Ky.; local, SW N. C. to N Fla. Lvs elliptic; ovary woolly; to dark purple. *V. woodii* Robbins 88,91,93,94,98,101,117-119,121,147; *V. intermedium* Chapm. 88,91-93,98,117.
- V. parviflorum* E USA: High elevation deciduous forest, Va. S to NE Ga., W into Tenn. and Ky. Lvs elliptic; ovary glabrous; stamen inserted well out on narrow green t; glands obscure. *V. parviflorum* Michx. 88,91-94,98,117,121; *Melanthium parviflorum* (Michx.) S. Wats. 118.
- V. taliense*. SW China: Edge of pine forest, mt of Yunnan and S Sikang. Like *V. parviflorum* but lvs linear; plant very large; glands definite. *V. taliense* Loes. 94,98, 146; *V. cavaleriei* Loes. 94, (98).
- V. mengtzeanum*. SW China: Dry meadows and pine or mixed forest, mt of Yunnan and S Sikang (and SW Kweichow?). Lvs linear; t large, obovate, thick, white; glands large, fleshy; ovary usu glabrous. *V. mengtzeanum* Loes. 94,98,146; *V. wilsonii* C. H. Wright ex Loes. 97,98,148.
- V. hybridum*. E USA: Open or rocky woods, in upland, Conn. and Pa. S to S. C. and Ga. Lvs oblanceolate; t white (turning green); slender claw bears stamen at or below middle; thick short obovate acuminate blade bears fan-shaped fleshy glands; ovary often mod pub. *V. hybridum* (Walt.) Zimmerman 94; *Melanthium hybridum* Walt. 91,93,118,121; *M. latifolium* Desr. 92,117.
- V. virginicum*. E USA: Moist Meadows and bogs, S New York to N Fla., W to E Texas, N to NE Iowa. Lvs linear; t white (turning green or reddish); slender claw bears stamen at or above middle; thick oblong-obovate blade bears oval fleshy glands; ovary often mod pub. *V. virginicum* (L.) Aiton 94; *Melanthium virginicum* L. 88,91-93,117-119,121,147,149; *M. dispersum* Small 92,117; *M. monoicum* Walt. 91.
- MELOVERATRUM. W Coast, USA: Mendocino and Sonoma Counties, Calif. Section *Meloveratrum* 94; Subgen. *Euveratrum*, Sect. *Alboveratrum* (97,98).
- V. fimbriatum*. T large, white, fringed; glands central, paired, large, fleshy; styles central; ovary sometimes pub.; capsule paper-thin, lobed, with sunken apex*; seeds few, large, green; leaves elliptic, sometimes pub.; ped divergent; 2° rac often long and branched; bulb large; rhizome short but stout. Marshes and shaded rivers on coast. *V. fimbriatum* Gray 91,93,94,98,100,101,120,124,128,129,134.

TABLE 2. *Continued*

- STENANTHIUM. C and N America; one reaching E Asia. T lanceolate-acuminate*; capsule $\frac{1}{4}$ – $\frac{1}{2}$ inferior; bulb; rhizome small or absent.
- EUSTENANTHIUM. E USA: Pa. to W Fla., W to E Texas and NW Mo.
- S. gramineum*. Slender; branches and fls many, the lower wholly staminate; t small, greenish to yellowish white; gland small, obscure; slender stolons* among roots; lvs linear. Moist meadows. *Stenanthium gramineum* (Ker) Morong 91, 92, 100, 117, 118, 121, 147, 150; *S. robustum* S. Wats. 91, 92, 100, 117, 147; *S. angustifolium* Kunth 93.
- STENANTHELLA. Pacific region. Fls few, large, bisexual; gland large, bilobed (obscure when dried).
- S. occidentalis*. W N America: Mossy stream banks in mt, W Mont. and N Calif. to Vancouver, B. C., and Banff, Alberta. E Asia: rocky places, Sakhalin. Small, slender; perianth greenish to reddish or purplish, campanulate; t tips reflexed*; sometimes a few branches; lvs often oblanceolate. *Stenanthella occidentalis* (Gray) Rydb. 88, 91, 125, 126; *S. sachalinensis* (F. Schmidt) Rydb.^d 88, 126; *Stenanthium occidentale* Gray, 93, 100, 127–130, 133, 134; *S. sachalinense* F. Schmidt 89, 93, 104, 115; *S. rhombipetalum* Suks. 151.
- S. frigida*. Mexico: Open pine forests in mts E and W of Mexico City. Stout to slender, often branched; t dark purple; lvs linear. *Stenanthella frigida* (C and S) Gates 91, *Stenanthium frigidum* (C and S) Kunth 88, 93, 139.
- ZYGADENUS. C and N America; one in Asia. By elimination of the other genera. Lvs linear; bulb (exc 1); rhizome small or absent (exc 1). (Note: The original spelling, *Zigadenus*, technically has priority; see 100, 152).
- AMIANTHIUM. E USA. To oblong, creamy white (to yellow or pink?), convexly narrowed at base, about as long as stamens; gland single, basal; ped long, crowded. Sect. *Oceanoros* (excluding *A. muscaetoxicum*) 152.
- A. muscaetoxicum*. Low sandy grounds, bogs, open woods, Fla W to S Mo. and Okla., N in mt to Pa. and on Coastal Plain to E N. Y. Rac unbranched; carpels broad, their tips separate*; seeds few, large, with fleshy reddish coat; t firm, turning green; gland obscure when dried; lvs rel broad, blunt. *Amianthium muscaetoxicum* (Walt.) Gray 88, 91, 93, 118, 121; *Chrosperma muscaetoxicum* (Walt.) Kuntze 92, 117.
- Z. densus*. Damp pineland and bogs, mostly on Coastal Plain, Fla. W to La., N to SE Va. Like *A. muscaetoxicum* but carpels very slender, their erect tips united up to the styles; seeds tiny, many; gland often visible when dried; lvs slender. *Zigadenus densus* (Desr.) Fern. 118, 121, 152; *Amianthium angustifolium* Gray 91, 93; *Tracyanthus angustifolius* (Michx.) Small 88, 92, 117.
- Z. leimanthoides*. Sandy pine-land and bogs, Coastal Plain and upland, local; C Ga. and W N. C. W to La. and N to Va.; N. J. and environs; E Texas. Like *Z. densus* but branched; gland thickened and distinct when dried. *Zigadenus leimanthoides* Gray 93, 118, 121, 152; *Amianthium texanum* (Small) Gates 91; *Oceanoros leimanthoides* (Gray) Small 88, 91, 92, 117.
- TOXICOSCORDION. Mt and plains of C and W N. America. T thin, white or yellowish, often narrowed to a short claw, usu about as long as stamens; gland single, central; ped long. Sect. *Chitonias* 152.
- Z. venenosus*. NW Mexico N to SW Canada. T usu shorter than 6 mm, and sometimes shorter than stamens; sepals acute to obtuse, clawed; infl elongate, seldom cymose, sometimes branched.
- venenosus*. Coast, Sierra Nevada, and Cascade Mts., from extreme NW Baja Calif. N to SE British Columbia, E in moist meadows to E Idaho. Ped ascend to spread; sepal claw about as well developed as petal claw; lower leaf sheaths usu lacking; exposed (upper) sheaths usu open, exposing slender stem; very rarely a branch or two. *Zigadenus venenosus* S. Wats. 100, 122, 125, 128–130, 134, 136, (127, 131), var. *venenosus* 133, 152; *Z. nuttallii* Gray (93); *Toxicoscordion venenosum* (S. Wats.) Rydb. 91, 125, 153; *T. arenicola* Heller 91; *T. salinum* (Nelson) Gates 91.
- gramineus*. Grassland and *Pinus ponderosa* forest, mostly NE of range of *Z. v. venenosus*, from Colo. and E Nebr. N to S Sask. and S British Columbia, and W to Cascade Mts. in Wash. Like *Z. v. venenosus* but sepal claw poorly developed (to 0.5 mm long); lower sheaths distinct; exposed sheaths elongate, enclosing the stout stem; sometimes a 2° rac or two. *Zigadenus venenosus* S. Wats. (127, 131), var. *gramineus* (Rydb.) Walsh ex Peck 130, 133, 152; *Z. gramineus* Rydb. 100, 126, 131, 132, (122); *Z. intermedius* Rydb. 126, 134; *Z. acutus* Rydb. 126; *Z. falcatus* Rydb. 126; *Toxicoscordion gramineus* Rydb. 119, 125, 153; *T. intermedium* Rydb. 88, 91, 153; *T. acutum* Rydb. 119, 125, 153; *T. falcatum* Rydb. 91, 125, 153.
- micranthus*. Serpentine and olivine hills, Klamath mts and NW Sierra and coast mt of Calif. Like *Z. v. venenosus* but ped sparser, longer and spread horizontally; t may be shorter than stamens. *Zigadenus venenosus* S. Wats. var. *micranthus*

TABLE 2. Continued

- (Eastw.) Jepson 128,129,152; *Z. micranthus* Eastw. 130,134; *Toxicoscordion micranthus* (Eastw.) Heller 91.
- fontanus*. Serpentine springs and marshes, C and W Calif. Like *Z. v. micranthus* but larger plant; t always as long as stamens. *Zigadenus venenosus* S. Wats. var. *fontanus* (Eastw.) Preece 152.
- Z. paniculatus*. Dry foothills, esp in sagebrush, from NW New Mexico and SW Mont. W to Cascade Mts. of Wash. and Ore. and to Sierra Mts. of Calif. and S Nev.; W into N-C Calif. Like *Z. venenosus* but sepals often acuminate and scarcely clawed; few to many 2° rac always present; plant usu large. *Zigadenus paniculatus* (Nutt.) S. Wats. 93,100,126-134,136-138,152,(122); *Toxicoscordion paniculatum* (Nutt.) Rydb. 91,125,153.
- Z. exaltatus*. Wooded W slopes of Sierra Mts in C Calif. Like *Z. paniculatus* but still larger plant with larger t (6-10 mm long). *Zigadenus exaltatus* Eastw. 128,129,134,152; *Toxicoscordion exaltatum* (Eastw.) Heller 91.
- Z. nuttallii*. Prairies, E Kans., Tenn. and S Mo. to S Texas. Like *Z. Venenosus* but claw indistinct on all t; rac usu cymose, sometimes branched; lvs stout, falcate. *Zigadenus nuttallii* Gray ex S. Wats. 118,121,126,149,152,(93); *Toxicoscordion nuttallii* (Gray) Rydb. 88,91,92,117,119,153; *T. texense* Rydb. 91,117.
- Z. brevibracteatus*. Mojave Desert, S Calif. T 6 mm or more long; ped very sparse, very long, spread horizontally from zig-zag axis; bracteoles up to 5 mm long; branched. *Zigadenus brevibracteatus* (M. E. Jones) Hall 128,129,134,152; *Toxicoscordion brevibracteatum* (Jones) Gates 91.
- Z. fremontii*. Slopes, esp in chaparral, on Pacific Coast and in coast mt from extreme NW Baja Calif. N to SW Ore. T very large (6-12 mm long), longer than stamens, with large gland; rac often branched. Variable. *Zigadenus fremontii* (Torr.) Torr. ex S. Wats. 91,93,100,128-130,134,152; *Toxicoscordion fremontii* (Torr.) Rydb. 88,153.
- ANTICLEA. C and N America; one in Asia. Generally at high alt and N lat. T thick, white to green, concavely narrowed to base, ascending to fruit; gland single, central, fleshy, bilobed; capsule about 1/2 inferior: ped mod long, sparsely spaced. Sect. *Anticlea* 152.
- Z. elegans*. N and C America, in dry to wet meadows, often near coniferous forest; t large (7-10 mm long), broad-ovate.
- elegans*. Western: NW Alaska and Yukon S to N Dak., S in mts to W Texas and NW Mexico. T usu yellowish white; rac often unbranched. *Zigadenus elegans* Pursh 88,100,112,114,118,121,125-127,130-134,137,138, var. *elegans* 152; *Z. coloradensis* Rydb. 91,125,126; *Z. mohinorensis* Greenm. 139; *Z. volcanicus* Benth. 137,139; *Anticlea elegans* (Pursh) Rydb. 91,119,153; *A. chlorantha* (Rich.) Rydb. 125,153; *A. glauca* Kunth 93; *A. alpina* (Blankinship) Heller 125; *A. gracilenta* (Greene) Gates 91; *A. longa* Heller, 91; *A. mohinorensis* (Greenm.) Gates 91; *A. coloradensis* Rydb. 153.
- glaucus*. Eastern: Gaspé Peninsula, Quebec, and S Ohio W to E N. Dak.; in mt of Va. and N. C. and S Mo. T more often greenish and bronze-tinged; rac usu branched; plant more often glaucous. *Zigadenus elegans* Pursh var. *glaucus* (Nutt.) Preece 152; *Z. glaucus* Nutt. 100,118,121,147; *Anticlea chlorantha* (Rich.) Rydb. 91,92,119. (Note: the type of *Z. chloranthus* Richards belongs with the western form, *Z. e. elegans*; see 118,152.)
- Z. vaginatus*. W USA: Wet sandstone, SE Utah. Like *Z. elegans* but t usu under 7 mm long, white. *Zigadenus vaginatus* (Rydb.) Macbride 152; *Anticlea vaginata* Rydb. 91,125.
- Z. volcanicus*. Alpine meadows, Guatemala. Like *Z. vaginatus* but t green-streaked; upper bracteoles longer; robust. *Zigadenus volcanicus* Benth. 152; *Anticlea volcanica* Baker 91,93.
- Z. sibiricus*. Asia: Open forests and rocky places; Siberian Arctic coast from 85° to 155° E long, S to Oiro, N Outer Mongolia, N Korea, Maritime Terr. (Far E USSR), and Riishiri I. (N Japan); C China (E Szechuan, W Hupeh). T narrowly ovate (1-3 mm wide) greenish, reflexed at anthesis; plant slender, often glaucous. *Zigadenus sibiricus* (L.) A. Gray ex Wats. 89,110,115,152; *Z. makinoanus* Miyabe and Kudo⁴ 104,105,152; *Anticlea sibirica* (L.) Knuth 91,93,153; *A. japonica* (Makino) Gates 91.
- Z. virescens*. Mt. forests; Mexico, Ariz., New Mexico. T ovate, small (5-7 mm long), on nodding ped. *Zigadenus virescens* (HBK.) Macbride 91,138,139,152; *Anticlea virescens* (HBK.) Rydb. 153; *A. porrifolia* (Greene) Rydb. 91,125,153; *A. mexicana* Kunth 93.
- EUZIGADENUS. SE USA on Coastal Plain, SE Va. to S Miss. Sect. *Euzigadenus* 152.
- Z. glaberrimus*. T thick, white, clawed, ascending in fruit; glands paired, central, fleshy; rac branched; ped sparse; bulb lacking*; rhizome elongate. Bogs, pineland. *Zigadenus glaberrimus* Michx. 91-93, 100,117,118,121,152,153; *Z. bracteatus* R and S 91.

TABLE 2. *Continued*

SCHOENOCAULON. C America and adjacent N and S America, in mt grassland, pine and oak woods, barrens, prairies, Rac spicate*, unbranched; fls often crowded; t ligulate to elliptic, very small, greenish; stamens often colored, usu exceeding t; gland usu obscure; lvs linear; bulb; rhizome small or absent.

GROUP I. T elliptic to ovate; margins finely denticulate.

S. drummondii. SE Texas from Bexar and Fayette Cos.; N Mexico. By elimination of others in group. *Schoenocaulon drummondii* Gray 154, (91,93,100,117); *Sabadilla drummondii* (Gray) B and R (88).

S. yucatanense. Mexico: Yucatan. Long filaments. *Schoenocaulon yucatanense* Brinker 154.

S. tenuifolium. Mexico: Oaxaca. Large t; seeds few, large; lvs rel broad. *Schoenocaulon tenuifolium* (Mart. and Gal.) Robins and Greenm. 139,154.

GROUP II. T ligulate, entire, but often with a pair of conspicuous hyaline-scarious flanges along part of their length.

S. comatum. Mexico: Oaxaca, Puebla, San Luis Potosi. Flange absent. *Schoenocaulon comatum* Brinker 154.

S. dubium. Florida. Flange absent; smaller than *S. comatum*. *Schoenocaulon dubium* (Michx.) Small 91,92,100,117,154; *S. gracile* Gray 93; *Sabadilla gracile* (Gray) B and R 88.

S. pringlei. Mexico: Hidalgo, D. F., Nayarit, Puebla. Flange extends along $\frac{2}{3}$ of t length; stamens barely exerted. *Schoenocaulon pringlei* Greenm. 139,154.

S. texanum. Mexico, S Texas (W from Travis and Bexar Cos.), SE New Mexico. One distinct hyaline tooth on each side. *Schoenocaulon texanum* Scheele 154; *S. drummondii* Gray (91,93,100,117); *Sabadilla drummondii* (Gray) B and R (88).

S. related species^d. Similar to *S. texanum*, differing slightly in size or shape of t, stamens, infl, or capsules 93,139,154. (*Schoenocaulon calcicola* Greenm.; *S. caricifolium* (Schlecht) Gray; *S. conzattii* Brinker; *S. coulteri* Baker; *S. intermedium* Baker; *S. jaliscense* Greenm.; *S. macrocarpum* Brinker; *S. megarrhiza* Jones; *S. mortonii* Brinker; *S. obtusum* Brinker; *S. regulare* Brinker; *S. tenue* Brinker).

S. ghiesbreghtii. Mexico: Chiapas. Two hyaline teeth or jags per side; faint t gland suggests relationship to Group III; filaments very long, curved. *Schoenocaulon ghiesbreghtii* Greenm.^d. 139,154.

GROUP III. T ligulate, entire; gland dark, near base, bilobed on sepal, smaller on petal; plant robust; lvs rel broad.

S. officinale. Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Peru, Venezuela. *Schoenocaulon officinale* (C and S) Gray 93,100,154; *Sabadilla officinale* (Schlecht) B and R 88.

Schoenocaulon is the most distinctive and homogeneous group, set off by its unbranched, spicate inflorescence, crowded flowers, very small tepals¹ and exerted stamens. Though unique in the tribe, this striking bottle brush-shaped inflorescence is shared by other liliaceous genera, such as *Chamaelirion* and *Chionocephalus* (88). A major variable among the species of *Schoenocaulon* is the tepal shape (fig. 5), of which the three main types are designated by the unnamed groups described in table 2. Brinker's monograph (154) suffered from insufficient herbarium material for unraveling the Mexican complex of entities related to *S. texanum* in Group II. More work is needed to determine which of these forms deserve specific rank.

The species of *Stenanthium*, in contrast, are few and diverse in aspect. The group is defined by its unique lanceolate, acuminate tepal shape (fig. 5) and by its partially inferior ovary. However, the latter feature (121, fig. p. 405) is shared with one group (*Anticlea*) of *Zygadenus* (121, fig. p. 408; 89, fig. 3b)⁵. Rydberg (126) erected a new genus, *Stenanthella*, for *S. occidentale*, because it differs in

⁴"Tepal" is a shorthand term for "perianth segment" in the lily family, where petals and sepals are very much alike.

⁵Many illustrations of the *Veratreae* are unreliable because of errors in labelling or in incomplete or incorrect drawing. For example, the good drawing of *Melanthium hybridum* in Small (92) is referred to as *M. virginicum*, while the drawing of *M. hybridum* in Gleason (121) omits the prominent glands and is placed less closely to the name than is the drawing of *Veratrum viride* flowers.

appearance and habit (plant much smaller, flowers much larger with reflexed campanulate perianth) from the first-named species, *S. gramineum*. The third species, *S. frigidum*, was then placed in *Stenanthella* by Gates (91) because, in its large, uniformly bisexual flowers, it more closely resembles *S. occidentale* than it does *S. gramineum*. While the two *Stenanthellae* appear to be quite uniform, *S. gramineum* exhibits considerable variability (in flower color and size, leaf morphology, plant size, and flowering phenology). This variation appears to be partly geographically clinal (118,150) and partly locally bimodal (147).

Veratrum has been delimited from the rest of the *Veratreae* by the presence of pubescence (at least in the inflorescence) and by broadly-winged seeds (94). In the other genera, the plants are wholly glabrous and the seeds wingless or only slightly winged or tailed. Two partial exceptions were included in *Veratrum* on the basis of other shared features, the glabrous *V. anticleoides* because of its rhizome and the shapes of its ovary and its obscure gland, and the wingless-seeded *V. fimbriatum* because of its rhizome, broad leaves and pubescence. Although unique in their incurving filaments (149) and slender abrupt tepal claws, the two species of *Melanthium* were included in *Veratrum*, (*V. hybridum* and *V. virginicum* in table 2), not only because of their pubescence and winged seeds, but also because they form the climax in a progressive series in tepal shape, gland development, and stamen adnation peculiar to those species of *Veratrum* in which the stamen is inserted on the tepal a short distance away from the ovary. For this group, which well illustrates variation on a theme (fig. 5), the section *Telandrium* was erected (94).

Zygadenus, thus defined as what is left of the *Veratreae* after carving off *Veratrum*, *Schoenocaulon* and *Stenanthium*, is a heterogeneous grouping. It has received careful treatment by Preece (152), who presents good grounds, such as differing chromosome numbers, for treating the four sections as separate genera, according to the subgeneric section characteristics given in table 2. Though he did not include *Amianthium muscaetoxicum* in his study, his work points to the grouping under the name *Amianthium* of *A. muscaetoxicum* and the two species of his section *Oceanoros* (as done in table 2). It may also be pointed out that one of the supposed oddities which set *A. muscaetoxicum* apart (few, very large seeds) also appears (homologously or analogously) in two very diverse groups, in *Veratrum fimbriatum* and *Schoenocaulon tenuifolium*. Though the three *Amianthia* are more closely similar to each other than are the three *Stenanthia*, they are still very distinct species; their sympatry implies the presence of breeding barriers. They exhibit very little variability, with the possible exception of *Z. leimanthoides*.

Of the remaining *Zygadeni*, *Z. glaberrimus* is even more uniform and distinct (note lack of synonymy in table 2); hence it is easy to defend monotypic generic status for it. Its well-developed rhizome without a bulb is unique in the tribe; and it shares several features (tepal shape, texture, and glands) with diverse groups in *Zygadenus* and *Veratrum* (fig. 5). At the other extreme are the species clusters comprising sections *Anticlea* and *Toxicoscordion*. These groups are distinct from each other and from other groups, but within them most of the entities are very similar to each other, and the discontinuities are not always sharp. Because of geographic clines and frequent allopatry, some of them were reduced by Preece to infraspecific rank, the two confluent forms of *Z. elegans* and the four of *Z. venenosus*. One could go farther and consider all of the large-flowered *Anticleae* (*Z. elegans*, *Z. vaginatus*, and *Z. volcanicus*) to be on the borderline between species and subspecies. Similarly, in *Toxicoscordion*, one is almost tempted to consider a series of subspecific relationships among *Z. nuttallii*, *Z. venenosus*, *Z. paniculatus*, and *Z. exaltatus*, at least. However, little would be gained by thus further adding to the burdensome synonymy, since local variants (some named, some not) vastly complicate the actual picture, as Preece notes (152) under *Z. elegans* and *Z. venenosus*.

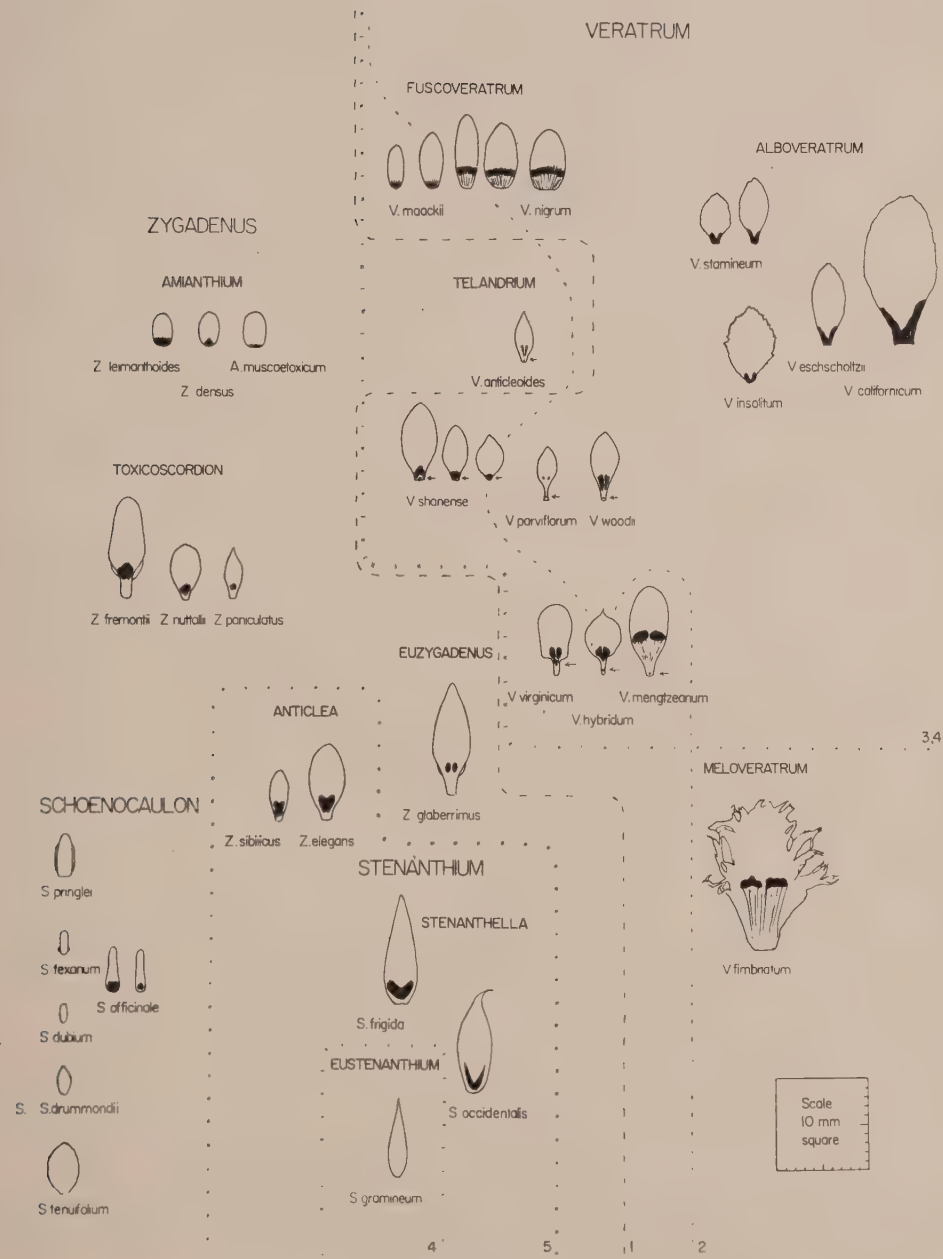


FIGURE 5

A summary of the botanical relationship between and within groups in the *Veratreae* should be as independent as possible of the practical but sometimes arbitrary generic categories used in naming them. Earlier attempts (91,97) at the ambitious task of relating the species in this tribe were often hampered by the lack of quantitative material for distinguishing large (or constant) from small (or variable) discontinuities between the species. The arrangement given in table 2 and in figure 5, which grew out of the need to define *Veratrum* (94), is based on a large sample, about 5,000 herbarium sheets, including all of the entities recognized in table 2 and almost all of the described species in the *Veratreae*; in addition, fresh material of a number of them was studied. The herbarium material examined, summarized in table 3, was distributed approximately as follows: *Veratrum*, 3,500 sheets; *Zygadenus*, 800; *Stenanthium*, 400; *Schoenocaulon*, 150.

TABLE 3. *Source material on which table 2 and figure 5 are based.*

Genus	Geographic Area	Herbaria Supplying Most of Material ¹
<i>Veratrum</i> (including <i>Melanthium</i>)	Europe	B DAO F G GH IA M MICH MO NY PH TENN US W WIS WU
	Asia	B CAL DAO E F FU G GH HK K LE M MO NA NY PH S SAP TNS UC US W WIS WU
	W North America	BRY COLO DAO F GH IA MICH MO MSC NA NO PH SMU UC UTC WIS
	E North America	DAO F FLAS GA GH IA MICH MO MSC NA PH SMU TENN UARK WIS
<i>Stenanthium</i> (including <i>Stenanthella</i>)	Asia	G GH LE NY
	Central America and W North America	F FLAS GH MICH MO MSC NA PH UTC WIS
	E North America	COLO F FLAS GA GH IA MICH MO MSC NA PH SMU TENN UARK WIS
<i>Zygadenus</i> (including <i>Amianthium</i>)	Asia	B CAL E F G GH K LE MO NY S TNS US
	Central America and W North America	F MO SMU TENN UTC WIS
	E North America	F FLAS MO MSC NA SMU TENN UARK UTC WIS
<i>Schoenocaulon</i>	Central America and North America	F FLAS MO NA WIS

¹The symbols are those of Lanjouw and Stafleu (158). Grateful acknowledgment is expressed to the curators of these herbaria for their generous loan of the *Veratreae* material.

In only a few instances (such as the ranges of some *Zygadeni* and *Schoenocaula*), was supplementary information added second-hand from the literature. The synonymy given in table 2 is based on examination of the same collections cited by the authors given, where possible, and otherwise where the geographic range or species description left no doubt of the identity.

The construction of figure 5 attempts to indicate the number of features shared by, and the number of discontinuities found between, each species or group and each of the others. Some degree of objectivity was attained by listing

in the squares of a correlation table an estimate of the number of differences observed between each possible pair among the representative species illustrated, and using those numbers to measure distances between the species before locating them on the figure. However, the subjective judgments required in weighing major against minor differences undoubtedly were an important source of bias. In addition to this necessary compromise between objectivity and experience, the limitations of a two-dimensional figure contributed to distortion. For example, in the correlation table, *V. fimbriatum* was actually found to be closer to section *Fuscoveratrum* than to section *Alboveratrum*.

A further handicap is the sole reliance on gross morphological features. Comparative anatomical work, such as that of Youngken (101,123) needs to be extended in order to test these relationships on the microscopic level. The features chosen for constructing fig. 5 were the types or relative development of: underground parts (rhizomes, bulbs, fibrillose leaf-bases); leaf shapes and leaf-reduction series; pubescence of inflorescence and leaves; branching of inflorescence and length and position of pedicels; distribution of pistils; tepals (size, shape, color, margin, gland, position); stamen-tepal adnation, length of filament, and time of opening and falling of anther; ovary shape; seed shape; adnation of perianth to ovary; and unique features.

These features may be plotted on the figure as it now stands. For example, large succulent tepal glands occupy the region from *Zygadenus* section *Anticlea* to *Veratrum mengtzeanum* and *V. fimbriatum*. Clines in the shape, position and development of the tepal glands can be traced in several directions—starting from *V. shanense*: (a) to section *Amianthium*; (b) to section *Toxicoscordion*; (c) to section *Fuscoveratrum* and, through section *Telandrium*, to *V. fimbriatum*; and (d) also through *Telandrium* to *Z. glaberrimus*, *Z.* section *Anticlea*, *Stenanthella*, and even to *Schoenocaulon officinale*. Similar relationships may be observed in tepal shape, such as the similarity of *Amianthium* to *Fuscoveratrum*, and the difference between their oblong tepal shape and the type which narrows concavely near the base, found in sections *Alboveratrum*, *Meloveratrum*, *Telandrium*, *Euzigadenus*, *Anticlea*, and *Toxicoscordion*. Another feature shared by adjacent species in the figure is the truncate type of ovary, well developed in section *Fuscoveratrum* and most of section *Telandrium*.

In certain other features one can think of the figure as a spindle-shaped continuum formed between two very unique groups each placed a little apart from the rest: At one end is section *Alboveratrum*, with stout rhizomes, broad pubescent leaves, tall stout leafy stems, pubescent and much-branched racemes, large tepals, functional pistils confined to the upper racemes, and numerous broadly-winged seeds. At the opposite end is genus *Schoenocaulon*, with bulbs alone, glabrous linear basal leaves, slender glabrous spicate inflorescence, much-reduced tepal size, uniformly bisexual flowers, and relatively few unwinged seeds. Each of these features changes somewhere between these poles, among the heterogeneous central groups of *Veratrum*, *Stenanthium* and *Zygadenus*, as a few lines drawn in figure 5 suggest. There are, of course, some irregularities in the gradient. The leaves of *Stenanthella occidentalis* tend to be oblanceolate. Functional pistils are found in almost all the flowers in *V. fimbriatum* and *V. maackii formosanum*, while they are lacking on the lower branches in *Stenanthium gramineum*, and often in *Z. leimanthoides* and *Z. paniculatus*. Persistent fibrillose leaf-bases curiously are most conspicuous at the two ends, in *Schoenocaulon* and *Veratrum*. It is hoped that this figure may be useful as a basis for further refinement as additional information comes to light.

COMPARISON BETWEEN ALKALOID CONTENT AND BOTANICAL TAXONOMY OF THE VERATREAE

It is evident from table 4 that only a small proportion of the plants which belong to the *Veratreae* have received phytochemical study. Furthermore, it must be

stressed that some of the plants which do appear in table 4 have received very little chemical investigation. Consequently, the absence of a report of isolation of a given alkaloid from a given plant should not necessarily be taken as evidence for the absence of the alkaloid from the plant. Considerable additional phytochemical work will be necessary before any appreciable number of firm chemical taxonomic correlations will be possible. For the present, one can only formulate some preliminary generalizations. It is hoped that these generalizations will point the way to additional phytochemical studies designed to further evaluate the potential significance of alkaloid occurrence in chemical taxonomy [cf. (159)].

TABLE 4. *Distribution of alkaloids isolated from the tribe Veratreae*

Plant name used in this paper	Jerveratrum alkaloids	Ceveratrum alkaloids	Unclassified alkaloids
<i>Veratrum album album</i> (including var. <i>lobelianum</i>).....	II;III;IV; XIII.	XVII;XXII;XXIII;XXIV; XXVII;XXXI;XXXIII; XXXIV;XXXVI;XXXVII.	XLII;XLIII; XLIV;XLV;XLVI; LIV;LVII.
<i>V. album oxysepalum</i> ...	I;II;IV.	XIV;XVII;XXXVIII.	
<i>V. album grandiflorum</i> ..	I;II;IV.	XIV;XLI.	
<i>V. viride</i>	I;II;III;IV; XI;XIII.	IX;XVII;XXIII;XXXIV; XXVII;XXVIII;XXIX;XXX; XXXI;XXXII;XXXIII; XXXIV;XXXVII.	XLVIII;XLIX;L; LI;LIV.
<i>V. eschscholtzii</i>	I;II;III;IV; XI;XII;XIII.	XVII;XXIV;XXXV.	
<i>V. stamineum</i>	I;IV.	XV.	
<i>V. fimbriatum</i>	IV;XIII.	XVII;XXIV;XXV;XXVI.	
<i>V. nigrum</i>	IV.	XXVII.	
<i>Amianthium muscaetoxicum</i>	IV.		XLVII.
<i>Zygadenus venenosus venenosus</i>		V;IX;XIV;XVI;XVII;XXIV; XXVIII;XXIX;XXXII.	
<i>Z. venenosus gramineus</i> .		V.	
<i>Z. paniculatus</i>		XIV;XVI;XVII;XXIV;XXIX.	
<i>Schoenocaulon officinale</i> .		VI;VII;VIII;XVIII;XIX;XX; XXI;XXXIX;XL.	LII;LIII;LV;LVI.

The most significant generalization apparent from the data assembled herein is that the alkaloid studies to date strongly support the botanical classification made along classical lines. The fact that *Veratrum* and *Schoenocaulon* elaborate different alkaloids (table 4) is entirely in accord with the wide botanical separation between the two genera (see fig. 5). Assignment of the genus *Zygadenus* to a position intermediate between *Veratrum* and *Schoenocaulon* is supported by a number of considerations. Thus, while all members of the genus *Veratrum* studied to date elaborate low-oxygen jerveratrum alkaloids as well as high-oxygen

ceveratrum alkaloids, *Zygadenus* and *Schoenocaulon* appear to elaborate only ceveratrum derivatives. Careful paper chromatographic analysis of the mixed alkaloids from *Z. venenosus venenosus* in this laboratory (with S. D. Levine) indicated the absence of jerveratrum alkaloids. *S. officinale* is one of the few plants of the tribe which has received extensive scrutiny in many laboratories; in no case has the presence of jerveratrum derivatives been reported. It has been noted above that zygadenine and germine esters have been isolated both from *Veratrum* and *Zygadenus* species. The relative proportion of zygadenine esters appears to be higher in *Zygadenus* species than in *Veratrum* species. In view of the "hybrid" chemical nature of zygadenine and its esters, discussed above, the relatively high zygadenine ester concentration in *Zygadenus* represents another factor which supports the intermediate taxonomic position assigned to *Zygadenus*. Apparently, no protoverine derivative has been isolated to date from *Zygadenus*, and one might be tempted to speculate as to the possible significance of the latter fact. However, the exceedingly close relationship between the structures and physical properties of germine and protoverine derivatives lead us to feel that it is likely that protoverine derivatives may occur in *Zygadenus* and may be isolated as more intensive phytochemical studies are undertaken.

A second preliminary generalization which characterizes the data summarized in table 4 concerns the nature of the 5-carbon acids in the ceveratrum ester alkaloids of different plants. Within the relatively small sampling of species and subspecies included in table 4, certain plants elaborate ester alkaloids containing only mono- or dihydroxymethylbutyrate residues, while others elaborate only angelate or tiglate esters. Thus, *V. album album*, *V. viride* and *V. nigrum* have yielded ester alkaloids which contain 2-hydroxy-2-methylbutyrate and 2,3-dihydroxy-2-methylbutyrate residues but none with angelate or tiglate residues.⁶ On the other hand, *V. album grandiflorum*, *V. eschscholtzii*, *V. stamineum*, *V. fimbriatum* and *S. officinale* have yielded angelate and tiglate esters, but no esters of the hydroxylated methylbutyric acids. It may be noteworthy that the former group consists of species which grow in areas adjacent to the Atlantic Ocean, whereas the latter group occurs in areas bordering the Pacific Ocean. This information, if substantiated by further work, may prove useful in tracing the origin, evolution, and migration of the *Veratreae*. Because of the small sampling examined to date, it must be emphasized that any generalization must be qualified, pending the accumulation of more data. Nevertheless, it would seem worth while to further examine the possibility that the apparent difference noted is a significant factor of potential taxonomic value. It is of incidental interest to note, in this connection, that the angelate ester, escholerine (XXXV), has thus far been isolated only from *V. eschscholtzii*, and that angeloylzygadenine (XV) has thus far been isolated only from *V. stamineum*. Also, *V. fimbriatum*, assigned a unique position in the botanical classification (fig. 5), elaborates two unique angelate esters, germanitrine (XXV) and germinitrine (XXVI, a monoangelate monotiglate).

Finally, the occurrence of jervine in *A. muscaelotoxicum* may be noteworthy. Classical taxonomy has assigned *Amianthium* to a position between *Veratrum* and *Zygadenus*, but a closer affinity to *Zygadenus* has generally been assumed. The fact that *A. muscaelotoxicum* elaborates jervine may suggest a closer proximity to *Veratrum* than heretofore believed. Perhaps the structure elucidation of amianthine (XLVII) and other alkaloid constituents of *Amianthium* will provide additional taxonomically useful data.

⁶An early paper reported that "cevadie" acid ("doubtless identical with tiglic acid") had been detected among the products of alkaline saponification of the alkaloids of *V. viride* (23). However, the saponification involved heating with alcoholic potash for twenty-four hours and distillation with dilute sulfuric acid, conditions which would undoubtedly lead to dehydration of 2-hydroxy-2-methylbutyric acid with consequent formation of tiglic acid (cf. footnote 25 in 17b.)

This survey has collected available data on the occurrence and structures of the veratrum alkaloids and on the classical botanical taxonomy of the *Veratreae*. The data have been examined seeking possible generalizations concerning the relationship between alkaloid content and botanical taxonomy of the *Veratreae*. It is apparent that, although the quantity of information concerning alkaloid occurrence is exceedingly small, certain preliminary patterns may be emerging. It is hoped that further phytochemical studies of the *Veratreae* may yield results which may be significant for their potential contribution to the understanding of the course of plant evolution, as well as for their taxonomic utility.

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Alkaloid Distribution in Colombian Cinchonas

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Cinchona bark, obtained from various species of the genus *Cinchona* (Fam. Rubiaceae) is noted in drug commerce for alkaloids contained therein. The four major alkaloids occur as two isomeric pairs: quinine and quinidine; cinchonidine and cinchonine. All of these are useful in the management of malaria, while quinidine has, in addition, a cardiac action. In areas where individual alkaloids are not obtainable, Totaquine (1) is used as an antimalarial. It is a mixture of alkaloids containing not less than 7 per cent quinine and not less than 70 per cent total crystallizable alkaloids.

Two other genera of the Rubiaceae are also known to contain "cinchona alkaloids," namely *Remijia* (2) and *Ladenbergia* (2). Barks of species of these generally contain a lower concentration of alkaloid.

The genus *Cinchona* of modern nomenclature was described by Linnaeus in *Genera Plantarum* in 1754, but had been known for its therapeutic value since 1633 (3). The natural range of cinchona is restricted entirely to South America, from the rainforests of Bolivia north to Colombia and Venezuela, on both eastern and western slopes of the Andes from elevations of 50 m to above 3500 m (2,4,5). *Cinchona* occurs in tropical to subtemperate well-drained upland slopes where the annual rainfall exceeds 85 inches per year, conditions which are found in the previously mentioned rainforests. Species of these vary from shrubs to trees up to 25 m in height and with trunk diameters over 1.1 m (2).

The problem of systematizing the genus has been sustained for many years as can be seen from the many attempts at a suitable classification (2,4-6). From these and other articles there is evident lack of agreement in the nomenclature of cinchona. In this paper, the authors have refrained from the use of specific names and have not attempted to evaluate existing names.

Through the activity of Charles Ledger, seeds of a high yielding type of cinchona were sold to the Dutch Government in 1865, marking the beginning of the era of the Dutch-Japanese cinchona monopoly (3). This industry grew to such proportions that in the first half of the twentieth century Java supplied 95 per cent of the world's cinchona bark. In the early years of World War II, the Japanese Government occupied Java, thereby eliminating the supply of cinchona to the allied countries. Thus in April, 1942, to the Board of Economic Warfare² fell the task of acquiring cinchona. The objectives of the cinchona missions established by this board were to obtain bark from available wild commercial stands and to form and develop a permanent cinchona plantation industry in the Western Hemisphere.

Agreements were negotiated with Colombia, Ecuador and Peru which gave the United States sole buying privileges for all barks above a minimum alkaloid level³. In turn, the United States Government was obligated to institute training and preparation of autochthones for the new cinchona industry.

Since Colombia was the first nation to ratify these agreements, operations were begun there at an earlier date than in the other countries. This resulted in a longer period of investigation, yielding a greater amount of data than in the other

¹This work was based on portions of a thesis submitted by L. C. Schramm to the Graduate School, The University of Connecticut, in partial fulfillment of the requirements for the M. S. degree.

²An agency in charge of procurement of strategic materials, later known as the Office of Economic Warfare and still later as the Foreign Economic Administration.

³The minimum level fluctuated from 2 to 3 per cent during the period of investigation.

countries. Colombia soon became the chief cinchona bark producer in South America. Other agreements elicited the establishment of plantations both in Costa Rica and Guatemala and the American Quinine Company's investigatory work in Venezuela.

In order to fulfill the primary objectives of this mission, that of a search for exploitable stands of cinchona, survey teams were organized. Each team usually consisted of a botanist, a forester and local assistants in training for future field work. The botanist identified and collected bark samples and, where possible, herbarium specimens. In Colombia, the procedure outlined in a manual by Fosberg (6) was followed. The forester was primarily interested in procurement, mensuration and harvesting procedures.

Alkaloid concentrations vary widely in *Cinchona* spp.; thus the only accurate method of establishing the acceptability of the bark was by means of chemical analysis. For this reason laboratories were established at Bogota, Colombia; Quito, Ecuador; Lima, Peru; and LaPaz, Bolivia. Their purpose was to assay the samples sent by the survey teams and the numerous commercial bark procurement organizations. These analyses facilitated rapid checks on area production, detection of adulterated or non-commercial barks and the determination of net worth of bark lot, since price was dependent upon alkaloid content.

The actual work of processing barks from harvest to shipment fell to agents contracted by the countries involved. These agents, under the guidance of the cinchona mission initiated production programs and established sub-agent buyers for the purchasing of barks at interior points.

The harvesting was accomplished by the cascarillero or quiñero⁴ who felled the trees and stripped or chipped the bark from the trunk and branches. Since most of the bark was removed by muleback, the bark was dried naturally or by artificial means in drying sheds near the place of collection. Cinchona bark loses up to 75 per cent of its weight in drying. Improperly dried bark or dry bark which subsequently becomes wet may show a loss in alkaloid content. After drying the bark was sampled and a portion of each sample was assayed for alkaloids to determine the net worth of the lot. The remainder of the sample was appropriately labeled with the original collector's number and the chronological assay number, and reserved. Bark specimens sampled for analysis fell into three categories: botanical or single tree specimens; commercial samples which were reasonably homogeneous; and export lots, composed of heterogeneous aggregates of commercial samples.

When the work of the cinchona mission was concluded in 1945, the records and bark specimens were shipped to Washington, D. C., and stored. In 1955, the School of Pharmacy, The University of Connecticut, contracted for the permanent loan of the data and specimens from the United States Government, Section of Plant Introduction, Horticultural Crops Research Branch, Agricultural Research Service, Plant Industry Station, Beltsville, Maryland.

In the course of preliminary examination of the records of the cinchona missions it was observed that because of the longer investigatory period, the Colombian mission had accumulated more data than the other missions. In fact, these data were more complete and in a better state of organization than that of the other missions.

This study was undertaken therefore, to evaluate the material obtained from the records of the Colombian cinchona mission. It was decided that a proper appraisal of the alkaloid distribution might be obtained by division of the total population into altitudinal and latitudinal segments to thus elucidate patterns or relationships. *Chemotypes*⁵ might be illustrated by modal analyses of the population in the geographical segments.

⁴Cinchona bark gatherer.

⁵A term used to describe the chemical make-up of an individual resulting from the interaction of genotypic characters and environment. Also, especially in this case, meaning a group of individuals sharing a specific chemotype.

EXPERIMENTAL DESIGN

The figures representing alkaloid concentration in cinchona bark were obtained from the files of the Bogota laboratory. Each bark sample used in compiling the data of this paper has been completely analyzed for alkaloids (7). The analyses were reported as follows: per cent anhydrous quinine plus cinchonidine (Q+C); per cent anhydrous cinchonine (CA); per cent anhydrous quinidine (QA); and total crystallizable alkaloids (TCA), represented by the sum of Q+C, CA and QA.

Geographical Considerations.—Colombia, geographically, is located on the northwest corner of South America. Two-thirds of Colombia is lowland plains with an altitude of less than 300 m, the major part of which lies in the eastern and southeastern portion of the country. The mountainous third defines an area crossing the country from north-northeast to south-southwest.

Traversing the mountainous area from west to east, three distinct ranges of mountains are encountered. They are termed respectively the Cordilleras Occidental, Central and Oriental. The Cordillera Central is separated from its neighboring ranges by the valley of the Río Cauca on the west and the valley of the

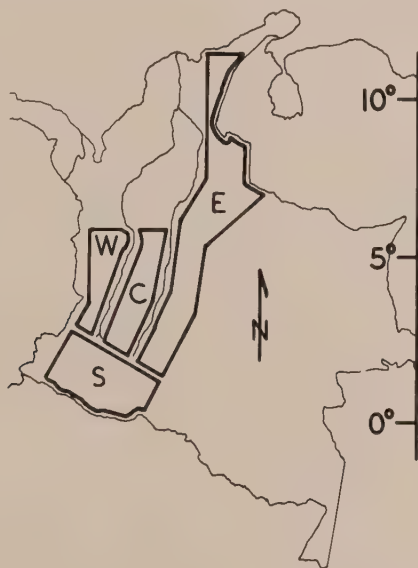


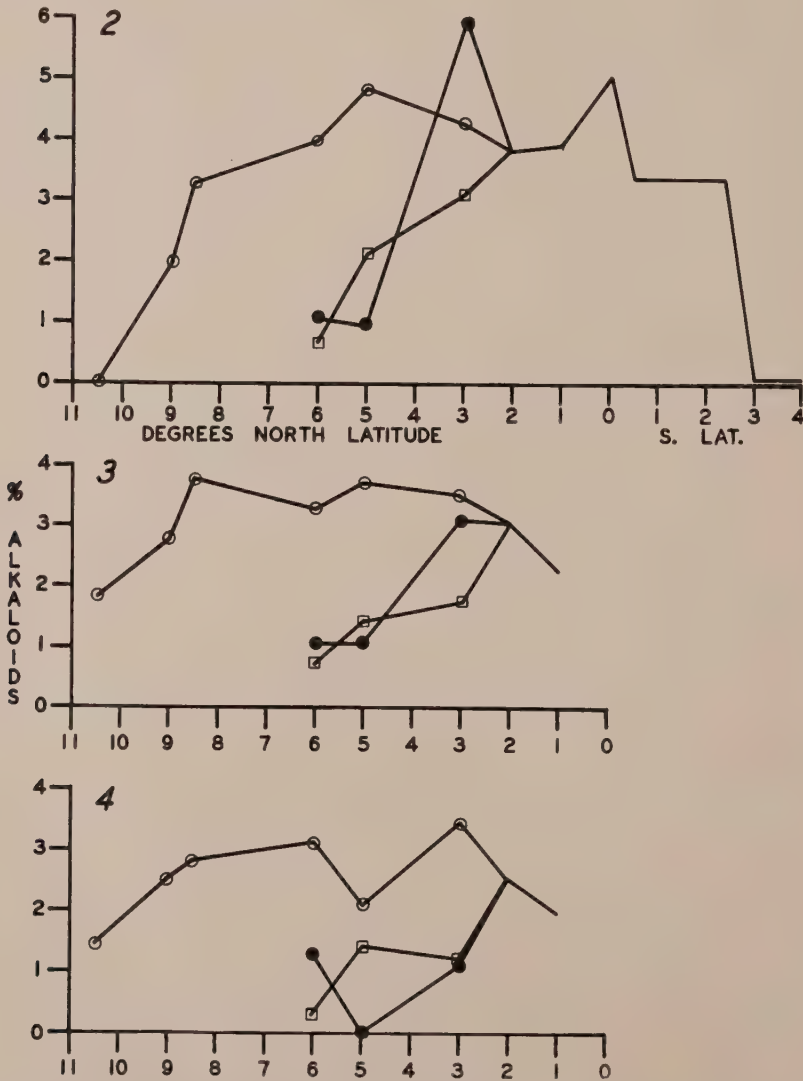
FIG. 1. Map of Colombia, South America, showing mountainous areas. W, western range (Cordillera Occidental); C, central range (Cordillera Central); E, eastern range (Cordillera Oriental); S, southern complex (Andean aggregate of southern Colombia).

Río Magdalena on the east. Both rivers drain northward into the Caribbean Sea. In the southern portion of this upland area the three Cordilleras merge toward the Cordillera Central, forming the main ridges of the Andes of southern Colombia and northern Ecuador. There are no distinct separations of cinchona habitats in this Andean aggregate of southern Colombia.

In order to determine the presence of probable chemotypes, the total population was divided as follows: eastern range sample, Cordillera Oriental, from the headwaters of the Río Magdalena northward to the sea; central range sample, Cordillera Central, from the headwaters of the Ríos Cauca and Magdalena northward to approximately 6° N lat; western range sample, Cordillera Occidental, from the headwaters of the Río Cauca northward to approximately 6° N lat; and southern complex, the Andean aggregate of southern Colombia, from the

headwaters of the Ríos Cauca and Magdalena southward to the Ecuadorean border (fig. 1).

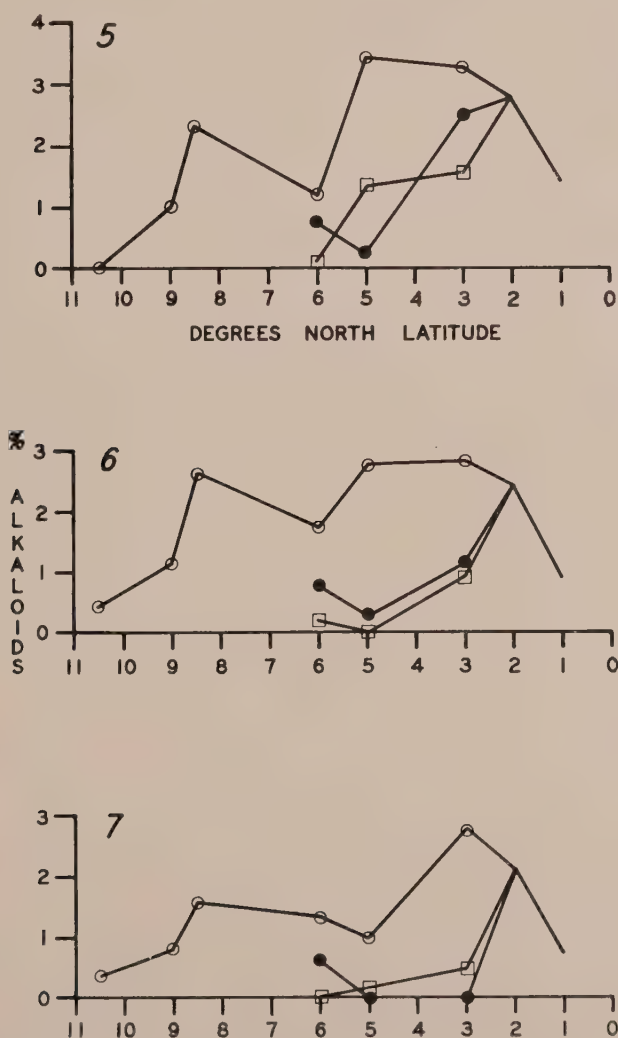
Mean Analyses.—The mean of a sample is defined as the sum of the individual values divided by the number of values. The data used in these analyses were derived from botanical or single tree specimens for which complete geographical and altitudinal information was available. Of the 4,000 specimens on file, 1,034 qualified for this study; 652 occurred in the eastern range sample, 129 in the central range sample, 127 in the western range sample and 126 in the southern complex.



FIGS. 2-4. Mean analysis, total crystallizable alkaloids (TCA). ○, eastern range; □, central range; ●, western range; area between 2° and 1° N lat, southern complex. FIG. 2. 2600-3200 m sample. The portion of this figure between 1° N lat and 4° S lat represents data reported by Camp (8). FIG. 3. 2000-2600 m sample. FIG. 4. 1100-2000 m sample.

Mean distributions were obtained for each of the four regions of Colombia by plotting alkaloid concentration on the ordinate and location in degrees N lat on the abscissa. Nine distributions were obtained in this manner. The population samples were analyzed for TCA, Q+C and CA at three altitude intervals. The results are recorded in figures 2-10.

Modal Analyses.—The mode of a distribution is defined as that value which occurs most frequently. The data used in these analyses were derived from botanical and commercial bark specimens for which complete alkaloid assays were available and for which sufficient geographical information was available to place the specimen in a specific geographical sample. Of the 4,000 specimens on file, 1,568 qualified for this study; 873 occurred in the eastern range sample, 218 in



FIGS. 5-7. Mean analysis, quinine plus cinchonidine (Q+C). ○, eastern range; □, central range; ●, western range; area between 2° and 1° N lat, southern complex. FIG. 5. 2600-3200 m sample. FIG. 6. 2000-2600 m sample. FIG. 7. 1100-2000 m sample.

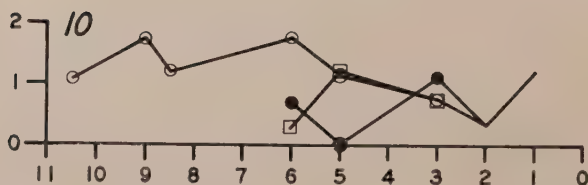
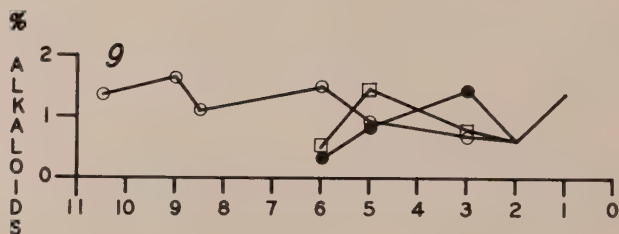
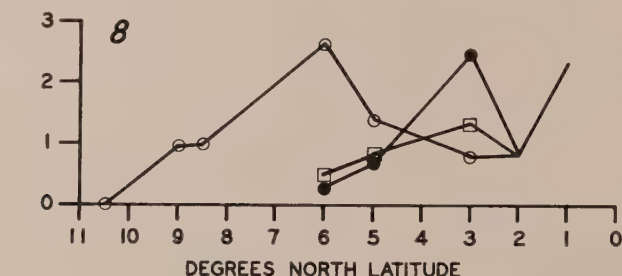
the central range sample, 204 in the western range sample, and 240 in the southern complex.

Modal distributions were obtained by plotting numbers of specimens on the ordinate and alkaloid concentration on the abscissa. Sixteen distributions were obtained in the above manner; the total sample and each range sample was analyzed for TCA, Q+C, CA and QA. The results of these analyses are recorded in figures 11-30.

EXPERIMENTAL RESULTS

MEAN ANALYSES

Total Crystallizable Alkaloids (figs. 2-4).—The alkaloid patterns of the three altitude intervals of the eastern range sample showed a lack of continuity from one interval to the other. TCA rose to a maximum of almost 5 per cent at 5°



FIGS. 8-10. Mean analysis, cinchonine (CA). ○, eastern range; □, central range; ●, western range; area between 2° and 1° N lat., southern complex. FIG. 8. 2600-3200 m sample. FIG. 9. 2000-2600 m sample. FIG. 10. 1100-2000 m sample.

N lat in the high altitude interval. In the intermediate interval TCA was near 4 per cent at 8.5° and 5° N lat but dipped slightly between these latitudes. In the low altitude interval, TCA dropped sharply at 5° N lat.

The central range sample possessed a pattern bearing similarity to the eastern range sample. The TCA value at 3° N lat was higher than the 5° value in both the high and intermediate altitude intervals, but the 3° value was lower than the 5° value in the low altitude interval.

The western range sample showed a transition from an S-shaped curve in the high altitude interval to a U-shaped curve in the low altitude interval. TCA fell from a high value of 5.75 per cent in the high altitude interval at 3° N lat to 0.0 per cent at the same latitude in the low altitude interval.

In the intermediate and low altitude intervals the southern complex TCA level decreased progressing southward. In the high altitude interval, however, the TCA level increased.

Quinine plus Cinchonidine (figs. 5-7).—These figures show the Q+C of the eastern range to parallel the TCA of the same range at 5° N lat. In the high altitude interval this value was the highest of all; in the intermediate interval, this value was approximately equivalent to the values at 8.5° and 3° N lat; in the low altitude interval this value was lower than the values at 8.5°, 6° and 3° N lat. Except for the high altitude interval, the pattern for Q+C was much the same as for TCA. All altitudinal intervals of the central and western range samples, with the exception of the high altitudinal interval of the central range, possessed quite similar patterns. In the exceptional case there was an increase in alkaloid content from 6° to 5° N lat. In all other cases there was a decrease.

In all altitudinal intervals of the southern complex the Q+C level decreased progressing southward.

Cinchonine (figs. 8-10).—The intermediate and low altitudinal intervals of the eastern range sample were similar in pattern. The high altitude interval was different in that the 6° N lat value of CA was much higher than the other values and the value for the northern end of the range was 0.0 per cent.

The pattern of the central range sample was the same in the intermediate and low altitude intervals. The high altitude interval showed a stepwise rise in CA from 6° to 3° N lat while the other intervals showed an initial rise to a high value at 5° then declined.

In the western range sample the pattern of the high altitude interval was quite similar to the intermediate interval. Both had a stepwise rise in CA level from 6° to 3° N lat while the low altitude interval had a value of 0.0 per cent at 5° N lat.

In all cases the southern complex CA level decreased progressing southward.

Quinidine.—Mean analyses were not performed on quinidine. Less than 6.4 per cent of the specimens yielded over 0.2 per cent making any such analyses erratic and nonconclusive.

MODAL ANALYSES

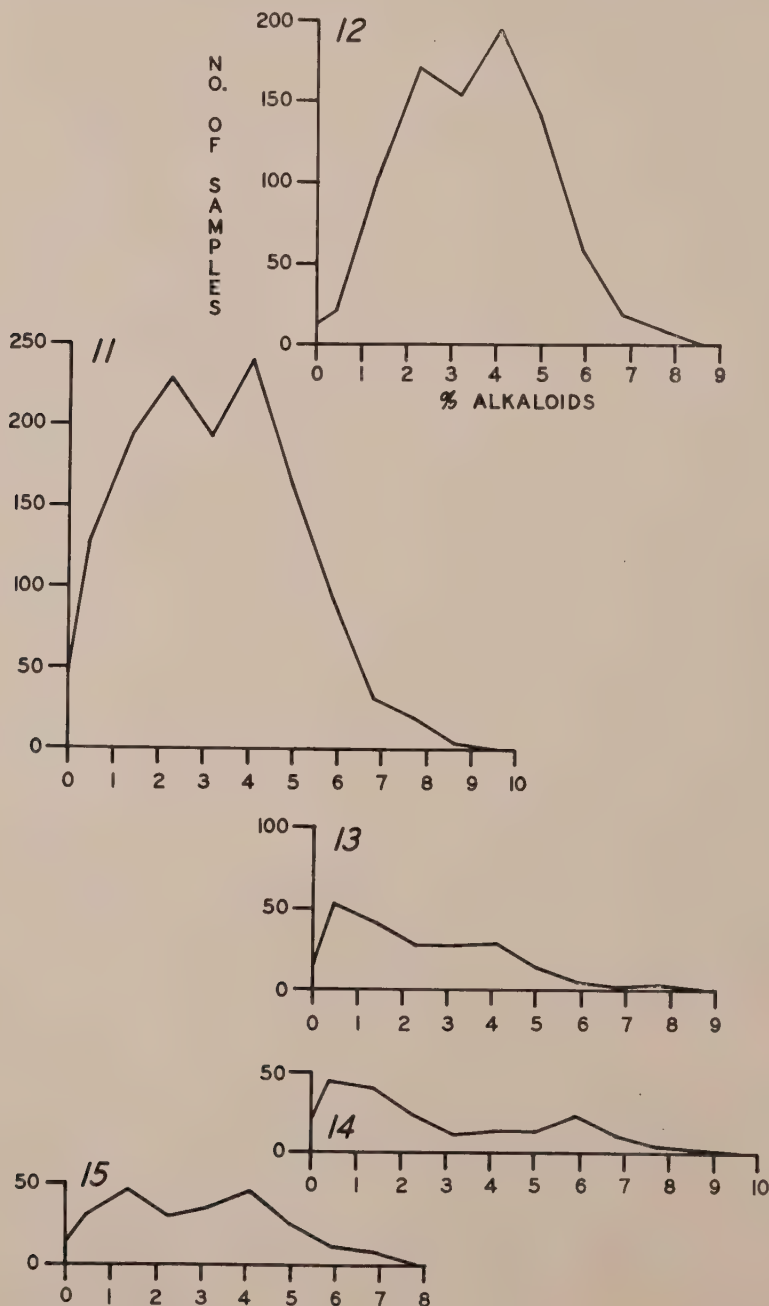
Total Crystallizable Alkaloids (figs. 11-15).—The total sample distribution presented a picture of bimodality with maximum values occurring at alkaloid concentrations of 2.3 and 4.1 per cent. If it were not for the break at 3.2 per cent this curve could be described as approaching a normal distribution. The actual distributions are perhaps positively and negatively skewed curves, the latter having a mode of 4.1 per cent.

The eastern range sample followed the total sample distribution, having two modes at the same values, 3.2 per cent and 4.1 per cent.

Although the central range sample distribution tended to be bimodal, it was not. The mode was 0.5 per cent, this value representing over 10 per cent of the samples.

The western range sample distribution had a mode of 0.8 per cent, a decline

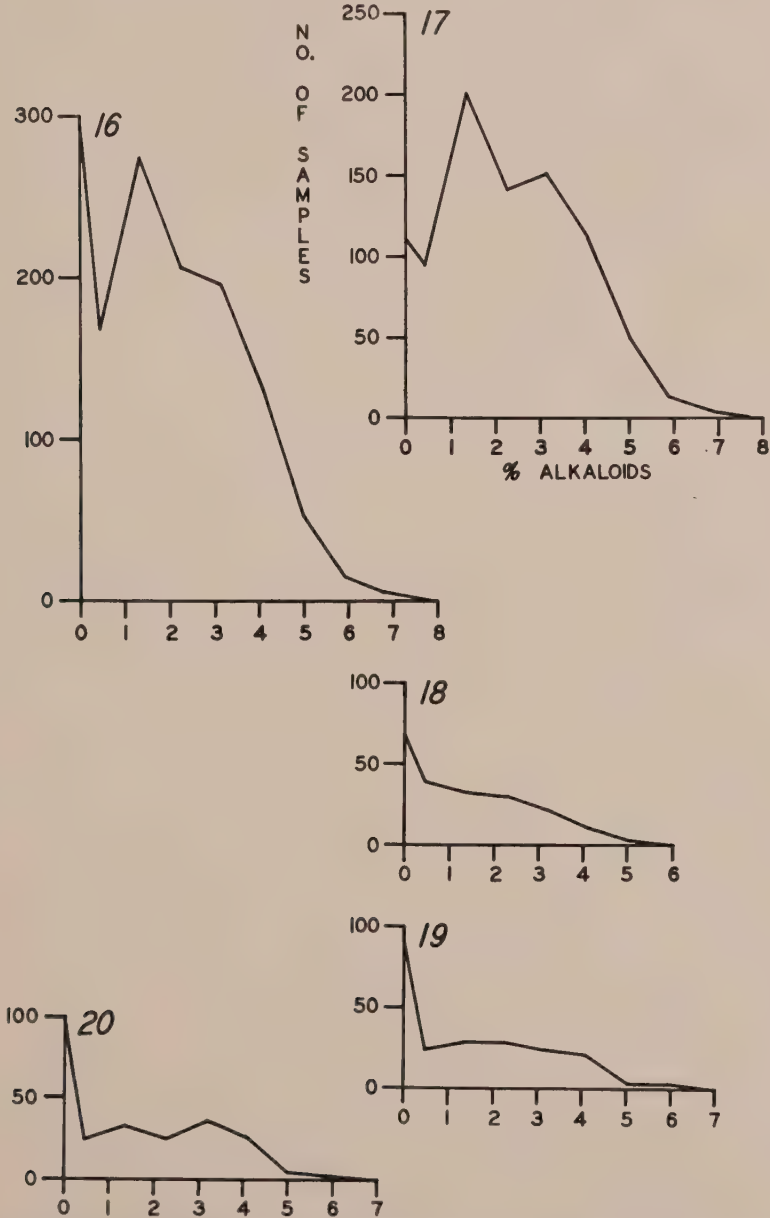
toward 3 per cent and finally a rise to a small maximum at 5.9 per cent. This latter value represented only 5 per cent of the specimens while the primary mode represented 10 per cent.



FIGS. 11-15. Modal analysis, total crystallizable alkaloids (TCA). FIG. 11. Total sample. FIG. 12. Eastern range sample. FIG. 13. Central range sample. FIG. 14. Western range sample. FIG. 15. Southern complex sample.

The southern complex followed the same bimodal pattern of the eastern range and total sample distributions. It was bimodal, the two modes being 2.3 and 4.1 per cent respectively.

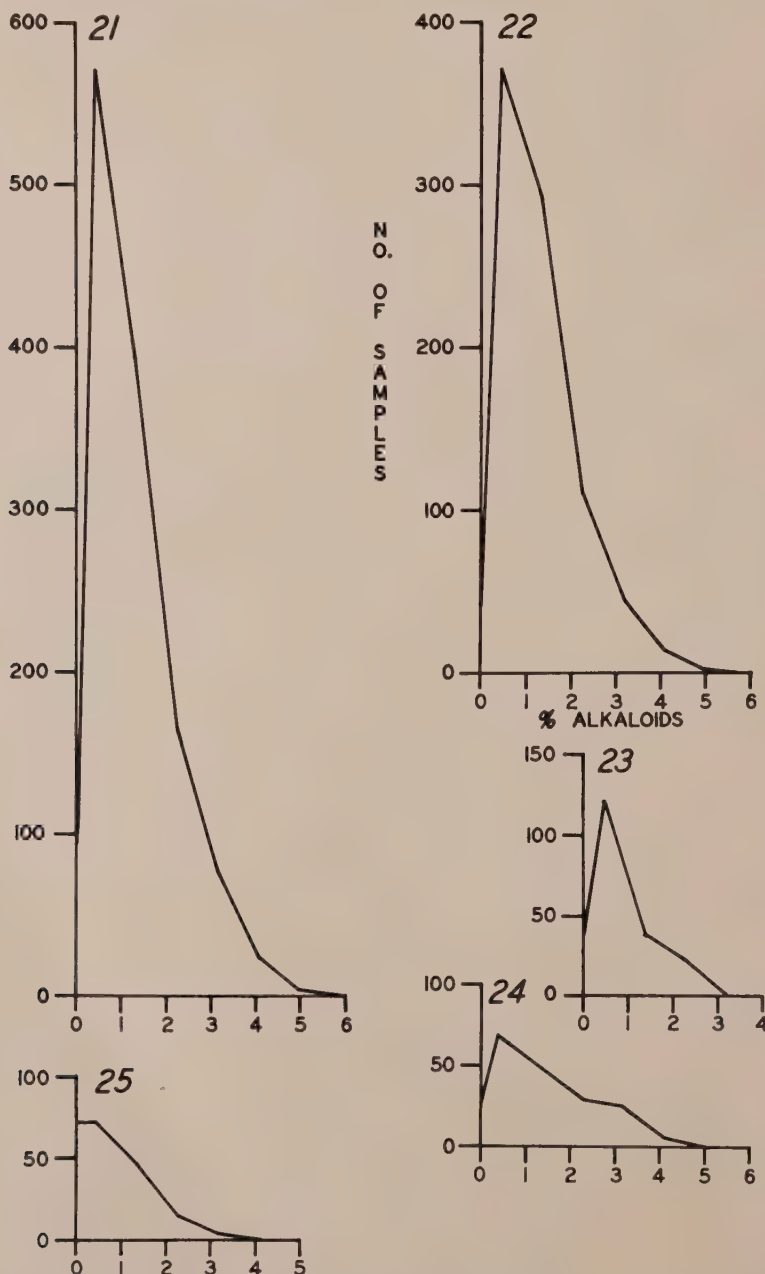
Quinine plus Cinchonidine (figs. 16-20).—The total sample distribution of figure 16 could be correctly termed bimodal. The two modes were 0.0 and 1.4 per cent. However, a shoulder occurred between 2.2 and 3.2 per cent. This



FIGS. 16-20. Modal analysis, quinine plus cinchonidine (Q+C). FIG. 16. Total sample. FIG. 17. Eastern range sample. FIG. 18. Central range sample. FIG. 19. Western range sample. FIG. 20. Southern complex sample.

shoulder was a "peak" in the eastern range sample distribution. There was a noticeable infrequency of samples containing 0.1 to 0.2 per cent Q+C.

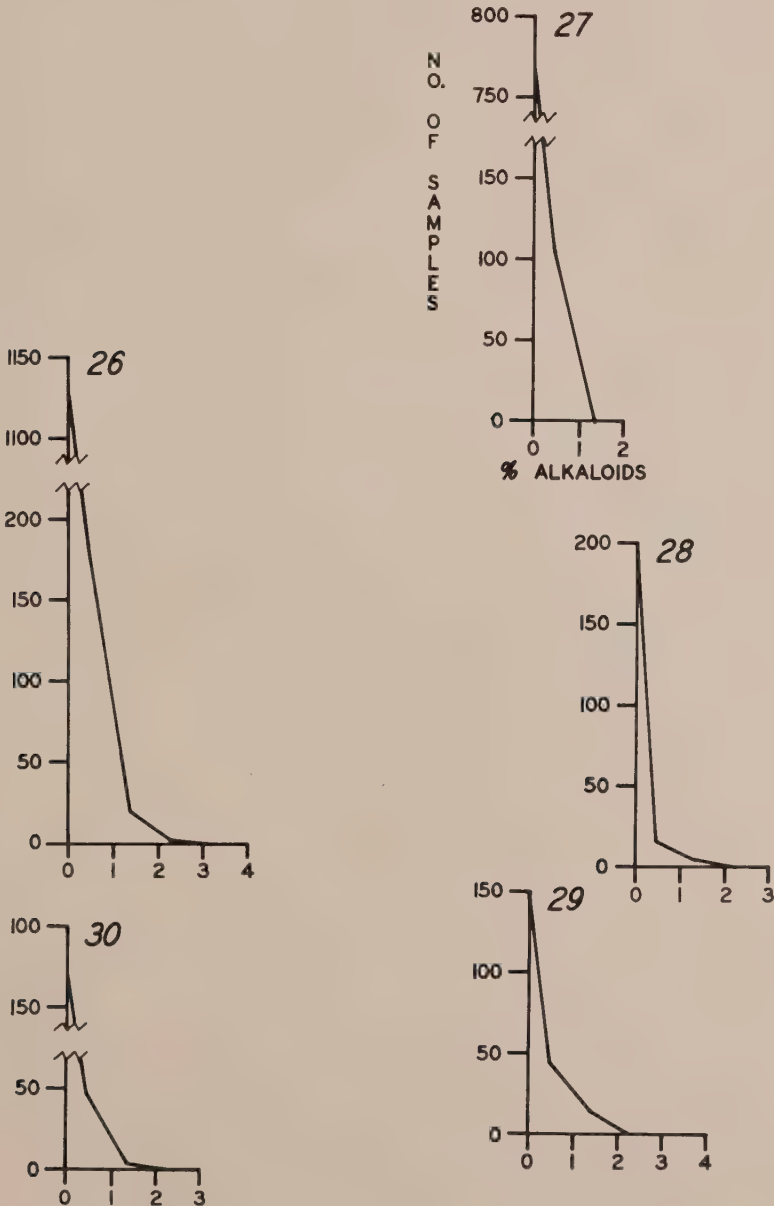
The eastern range sample distribution tended to develop the pattern of the total sample distribution. Here, a tertiary mode of 3.2 per cent occurred, and



FIGS. 21-25. *Modal analysis, cinchonine (CA)*. FIG. 21. *Total sample*. FIG. 22. *Eastern range sample*. FIG. 23. *Central range sample*. FIG. 24. *Western range sample*. FIG. 25. *Southern complex sample*.

fewer specimens yielded a value of 0.0 per cent. It would be possible to "synthesize" this distribution from three artificial curves, a highly leptokurtic, a moderately leptokurtic and a normal curve. Such a combination would form a trimodal system as occurred in this case.

The central range sample distribution differed greatly from the other distributions. This could be best described as an inclined straight line.



FIGS. 26-30. Modal analysis, quinidine (QA). FIG. 26. Total sample. FIG. 27. Eastern range sample. FIG. 28. Central range sample. FIG. 29. Western range sample. FIG. 30. Southern complex sample.

The western range sample distribution resembled the central range rather than the total or eastern range sample distributions. This curve, however, tended to show bimodality. The two modes were 0.0 and 1.4 per cent.

The southern complex distribution again paralleled the total and eastern range sample distributions. Here the distribution was trimodal, the modes being 0.0, 1.4, and 3.2 per cent respectively.

Cinchonine (figs. 21-25).—All distributions were approximately equivalent. All, with the exception of the southern complex, had a mode of 0.5 per cent and in this case the mode was 0.0 to 0.5 per cent. There were relatively more specimens yielding 0.0 per cent in this sample than in the other samples.

Quinidine (figs. 26-30).—All distributions were equivalent. The mode in each case was 0.0 per cent with no sample yielding over 3 per cent QA.

DISCUSSION

Although a maximum mean value of TCA of almost 5 per cent was reached in the central portion of the Cordillera Oriental (fig. 2), the values then declined toward the south. The high value of 5 per cent, reported by Camp (8) for southern Colombia, was not observed in that area in this work. Indeed, the location of Camp's samples was defined only as being "taken in series from the Colombian Ecuador border northward essentially to the known limits of *C. pitayensis* at about 3° N lat." From the present work, one might surmise that they were an extension of the 3° N lat western range population. This area is not typical of southern Colombia. The average TCA for extreme southern Colombia is just under 4 per cent, a value well below those of northern Ecuador, the southern portion of the western range and the 5° N lat portion of the eastern range. The sharp decline in TCA progressing northward in the central range suggests the presence of a low-yielding chemotype. Perhaps this low-yielder when in contact with the other type(s) would tend to bring down the TCA content. The southern complex is placed in the status of a meeting point of the Andean ridges and their plant populations.

In all mean analyses except the low altitude interval of the CA analysis, there was a tendency for the following pattern at the 5° N lat area: the high altitude interval was high in alkaloid content; the intermediate interval was moderate in alkaloid content; the low interval was low or lacking in alkaloid content.

In all the modal analyses, the eastern range sample, being the largest in quantity, contributed most to the total sample and thus always paralleled the total sample.

Uni-, bi- and trimodal patterns were apparent in these analyses. Unimodal patterns of chemical constituents in a plant population usually signify a single chemotype. A bimodal pattern would thus represent two chemotypes, or two types of individuals producing some chemical compound or mixture in different quantities.

The majority of samples used for these investigations were identified as either *C. officinalis*, *C. pubescens* or *C. pitayensis* (6). The first two species were quite abundant while the latter was found rather infrequently. A cursory scanning of the analysis sheets of certain regions indicated a pattern of three levels of alkaloid production. *C. pubescens* was usually associated with the lowest level, *C. officinalis* with the intermediate level and *C. pitayensis* with the highest level. The modal distributions did not portray this pattern as well as might be expected, with the exception of the eastern range and southern complex samples of Q+C (figs. 17, 20). The majority of the figures presented bimodal patterns. It is possible to "synthesize" this pattern by overlapping three normal curves of equal size. The sum of the three curves would produce two modes, precisely at the overlap points. The central and western ranges, whose sample size was less than one-fourth that of the eastern range, possessed relatively vague patterns.

Working with natural populations of plants from the viewpoint of chemical constituents, as with cinchona, certain important features become obvious. The natural population was obviously heterogenous. Alkaloid production in cinchona appeared to be genetically controlled, but there was no simple pattern of exclusion of one category of alkaloids for another. Cinchona alkaloids evidently exist independently of one another.

When concerned with any one specific alkaloid category, one phenomenon occurred repeatedly. The number of samples yielding 0.0 per cent stood alone. In the majority of cases, there was no figure at 0.1 per cent or even 0.2 per cent related to the 0.0 per cent category. Perhaps the analysts placed those samples which gave only a faint Grahe test in the 0.0 per cent category. These low-yielding barks were not commercially useful, and the analysis might not have been economically feasible. Nevertheless, the fact remains, that although a plant contains a principle in varying quantities, it does not mean that all individuals of a genus or species will contain the principle. The portion of a population possessing or lacking any certain principle or aggregate of principles varies widely.

Proper sampling of a population usually presents a good indication of the general distribution of individuals, whether they appear continuously throughout the area or occur in separated groups. The sampling of cinchona by the cinchona missions produced both expected and curious results. Compared with similar regions of the eastern range, both a paucity of samples and a lower alkaloid level were noted in parts of the central and western ranges. The specific reason for these phenomena cannot be determined with the present data. Some of the factors which in all probability influenced the distribution are presented.

The central and western ranges appear to have supported larger human populations in pre-Conquest times than at the present as evidenced by numerous artifacts discovered in both past and recent explorations. It has been noted that some of the wood used in the primitive buildings was cinchona wood; even today it is utilized as a construction material because of its termite-resistant qualities (8).

Due to variable amounts of moisture and winds at certain levels of the mountains, cloud strata occur in the Ecuadorean Andes (8). Cinchona was found primarily within these cloud belts, but often not between them. This occurrence was attributed by Camp (8) to the clearing of the more inhabitable intercalated drier zones by man for farms and dwellings.

Cinchona wood for construction purposes was obtained not only from the dry belt prior to or during clearing, but in some instances from the cloud belts. It is not impossible to infer that similar situations have existed in Colombia.

In some areas, cultivated crops have taken the place of cinchona forests. Manizales, in Caldas, is reputed to be the coffee capital of the world. Indeed, the major export of Colombia in recent years has been coffee. *Coffea* spp., also rubiaceous, grows in areas well suited to the growth of *Cinchona* spp. The higher elevations which may be suitable for cinchona are not especially favorable for coffee cultivation.

These are factors which tend to disrupt the cinchona population. They have been in effect for centuries, and probably will occur with more force and regularity in the future. When a wild population such as cinchona becomes divided, the groups, if isolated, may produce unusual progeny due to segregation, regression and progression of types.

Other factors, perhaps not influencing the distribution but the sampling of cinchona are attributed to natural and man-made causes.

Since the primary purpose of the mission into Colombia was the search for exploitable stands of commercially important cinchona trees, it may be reasonable to suspect that various commercially worthless barks may have been passed over. The survey teams were able to determine the worthless barks not only by observation of the morphological characters of the tree in question, but in many cases by the taste perception of the bark itself.

Colombia, and in fact much of South America, did not enjoy the transportation network during the war years which is so familiar to North Americans. Roads were infrequent and undependable; railroads existed only between river ports and the major cities of the interior; air transportation was in its infancy. The only other methods of transportation were on horse, on mule, or on foot. Indeed, the majority of bark samples were transported from their original sources by mule or horseback; some were carried at least part way on manback. Many areas of the mountains were either economically or physically inaccessible. It would have been economically impossible to carve roads through the forests to some areas in which commercial bark specimens were in abundance but scattered over a wide area. This physical inaccessibility is borne out by the fact that recent maps still list the major portion of Colombia in conjectural contour lines.

It cannot be inferred that these factors are the only ones influencing the cinchona population. When the complete geographical, geological and climatic forces and conditions have been described, and perhaps a more complete picture of the population size and distribution becomes available, logical explanations can be drawn with respect to the problem of morphological and chemical taxonomy.

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The Amino Acid Composition of some Ascosporic Members of the *Aspergillus nidulans* Group

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The occurrence and factors affecting the synthesis of amino acids of the fungi has been studied by a number of investigators. Thirteen amino acids were isolated from *Aspergillus sydowi* (1-3). Qualitative and quantitative differences in the amino acids and sugars of the uredospores of certain races of cereal rusts (4) and the effect of different carbohydrates on amino acid synthesis by *Aspergillus oryzae* has been reported (5). Two species of *Emericellopsis* were differentiated according to their amino acid contents (6).

γ -Amino butyric acid has been reported (7, 8) in the protein hydrolysate of mycelium of species of *Fusarium* and this substance has also been reported to occur in yeast (9), *Aspergillus flavus* (10) and *Penicillium chrysogenum* (11). Steward and Thompson (12) reported that this amino acid is not a constituent of protein.

The present investigation sought to determine the amino acids produced by five ascosporic species of the *A. nidulans* group when grown on defined media and to determine the effect of different carbohydrates on the capacity of one of them to synthesize amino acids. Consideration was given to the relationship between the occurrence of these acids and the taxonomic position of the individuals of the group.

MATERIALS AND METHODS

Cultures of the following species of Aspergilli were used: *A. nidulans* (Eidam) Wint.; *A. violaceus* Fennell and Raper; *A. varicolor*, (Berk. and Br.) Thom and Raper; *A. rugulosus* Thom and Raper, and *A. quadrilineatus* Thom and Raper. The fungi were grown in 150 ml Erlenmeyer flasks containing 20 ml of medium of the following composition: sucrose, 10.0 g; NaNO_3 , 3.0 g; KH_2PO_4 , 1.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; and 1000 ml distilled water. Five replicates were made in each case. The flasks were inoculated with the different species by seeding ascospore suspensions on the surface of the medium. Care was taken to insure that almost equal quantities of spores were used. Inoculated flasks were incubated at room temperature ($25^\circ\text{C} \pm 2$) for 16 days. After the incubation period the mats were harvested and dried. The free amino acids were obtained by extracting the dry mycelium with 80 per cent ethanol and the protein amino acids by hydrolysis of the extracted mycelium with 6 N hydrochloric acid (13). Both fractions were analyzed by a two dimensional chromatographic method (14).

The chromatograms were visually compared with each other to relate size, color, and intensity of the spots. This method has been followed by other investigators (8, 15).

To determine the effect of different carbohydrates on the amino acid synthesis in Aspergilli one common species, *A. nidulans*, was used. Various carbohydrates were substituted for sucrose in the basal medium to supply 4.21 g of carbon per liter in each case. The amount of starch added was the same as that of sucrose. All the experiments were repeated two or three times.

RESULTS

Free Amino Acid Composition.—The amino acid composition of the ethanol soluble fraction of the mycelium of different Aspergilli is given in table 1. The following free amino acids were found in the mycelial extract of all the species: aspartic acid, glutamic acid, glycine, serine, alanine, arginine, glutamine, valine,

leucine and isoleucine. Histidine was present in every case except *A. varicolor*. γ -Amino butyric acid was found only in *A. nidulans*. Of the aromatic amino acids phenylalanine was detected in *A. nidulans*, *A. rugulosus*, *A. violaceus*, and *A. quadrilineatus* and tyrosine in *A. nidulans*, *A. varicolor* and *A. rugulosus*. Of the sulphur containing amino acids cysteic acid was detected only in traces in *A. quadrilineatus*.

TABLE 1. *The free amino acid composition of the mycelium of different Aspergilli.*

Amino acid	<i>A. nidulans</i>	<i>A. rugulosus</i>	<i>A. violaceus</i>	<i>A. varicolor</i>	<i>A. quadrilineatus</i>
Aspartic acid....	++ ¹	++	++	++	++
Glutamic acid....	++	++	++	++	++
Serine.....	+	+	+	+	+
Glycine.....	+	+	+	+	+
Alanine.....	++	++	++	++	++
Arginine.....	+	+	+	+	+
Histidine.....	+	++	+	+	+
Glutamine.....	++	++	++	+	+
γ -Amino butyric acid.....	+	—	—	—	—
Valine.....	+	+	+	+	+
Isoleucine.....	+	+	+	+	+
Leucine.....	+	+	+	+	+
Tyrosine.....	+	+	—	+	—
Cysteic acid.....	—	—	—	—	+
Phenylalanine....	+	+	+	—	+

¹(+) and (++) indicate relative amount; (—) indicates absence.

TABLE 2. *The bound amino acid composition of the mycelium of different Aspergilli.*

Amino acids	<i>A. nidulans</i>	<i>A. rugulosus</i>	<i>A. violaceus</i>	<i>A. varicolor</i>	<i>A. quadrilineatus</i>
Aspartic acid....	++ ¹	++	++	++	++
Glutamic acid....	++	++	++	++	++
Glycine.....	+	+	+	+	+
Serine.....	+	+	+	+	+
Alanine.....	++	++	++	++	++
Threonine.....	—	—	+	+	+
Tyrosine.....	+	+	+	+	+
Arginine.....	+	+	+	+	+
Histidine.....	+	+	+	—	+
Proline.....	+	—	+	+	+
Valine.....	+	+	+	+	+
Leucine.....	+	+	+	+	+
Isoleucine.....	+	+	+	+	+
Cysteic acid.....	+	+	—	+	+
Phenylalanine....	—	—	+	—	—

¹(+) and (++) indicate relative amount; (—) indicates absence.

Bound Amino Acid Composition.—The bound amino acids in different *Aspergilli* are recorded in table 2. Proline and threonine which were absent in the soluble fraction were observed in the insoluble fraction. The former was found in all species except *A. rugulosus* and the latter was present in *A. varicolor*, *A. violaceus* and *A. quadrilineatus*. Tyrosine which was not found in the soluble fraction of *A. violaceus* and *A. quadrilineatus*, was detected in the insoluble fraction of these two species. Cysteic acid which was present only in the soluble fraction of *A. quadrilineatus* was detected in the insoluble fraction of all species except

A. violaceus. Glutamine which was found in the free form was absent in the insoluble fraction in all cases.

Effect of Different Carbohydrates.—The results of the experiments on the effect of different carbohydrates on the free amino acid composition of *A. nidulans* are given in table 3. The data of table 3 and that derived from table 1 indicate that certain amino acids occur in the mycelium of *A. nidulans* irrespective of the carbon source. These are aspartic acid, glutamic acid, serine, histidine, glutamine, arginine, and valine. Glycine was not found in mycelium grown on media containing glucose, fructose, and raffinose. Alanine was not found when the growth in media contained galactose and leucine and isoleucine were not observed when the growth media contained raffinose and starch. Proline was detected only when the organism was cultured in medium containing glucose. γ -Amino butyric acid was found in the mycelium harvested from media containing glucose, raffinose, and starch, while cysteic acid was found only in the mycelium of fructose medium.

TABLE 3. The free amino acid composition of the mycelium of *A. nidulans* grown on different carbon sources.

Amino acids	Glucose	Fructose	Galactose	Lactose	Raffinose	Starch
Aspartic acid.....	+	+	+	+	+	+
Glutamic acid.....	+	+	+	+	+	+
Serine.....	+	+	+	+	+	+
Glycine.....	—	—	+	+	—	+
Alanine.....	+	+	—	+	+	+
Glutamine.....	+	+	+	+	+	+
Histidine.....	+	+	+	+	+	+
Arginine.....	+	+	+	+	+	+
Valine.....	+	+	+	+	+	+
Leucine.....	+	+	+	+	—	—
Isoleucine.....	+	+	+	+	—	—
Cysteic acid.....	—	+	—	—	—	—
γ -Amino butyric acid.....	+	—	—	—	+	+
Proline.....	+	—	—	—	—	—

SUMMARY AND CONCLUSIONS

The amino acids of the mycelium of five ascosporic species of the *Aspergillus nidulans* group grown in a synthetic medium were determined with the use of two dimensional paper chromatography. The results show that aspartic acid, glutamic acid, glycine, serine, alanine, arginine, valine, leucine, and isoleucine were found in either free or bound form. Proline and threonine were detected in bound form only.

The frequency of occurrence of certain amino acids is noteworthy. Tyrosine is absent in the free amino acid fraction of *A. violaceus* while histidine is absent in *A. varicolor* in both free and bound form. These and other differences in the amino acid content of the different species grown on a basal medium containing sucrose have been found and can be used to differentiate among the *A. nidulans* group.

The effect of different media carbohydrates on the synthesis of amino acids by *A. nidulans* (Eidam) Wint. was investigated. Free leucine and isoleucine were found in mycelium grown on media containing sucrose, glucose, galactose, fructose, and lactose and these acids were absent when the growth medium contained raffinose and starch. Glycine was absent in cultures grown in media containing glucose, fructose and raffinose but was present when the organism was grown in media containing sucrose, galactose, lactose and starch. These and other data further emphasize physiological differences which are useful as taxonomic criteria.

ACKNOWLEDGMENT

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A Study of the Alkaloids of *Thalictrum*. I. Isolation of some Quaternary Alkaloids from *Thalictrum dasycarpum* var. *hypoglaucom*

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In recent years increased interest has been shown in the genus *Thalictrum* by several investigators in different parts of the world. The Willaman and Schubert (1) study revealed that of 62 species of Ranunculaceae investigated, 58 gave evidence of containing alkaloids. The Ranunculaceae to which the genus *Thalictrum* belongs, is composed of 1,500 species. *Thalictrum* is a widely distributed and abundant genus (3-5). By 1942 approximately 165 species had been proposed to designate the American *Thalictra* (6). One hundred fifteen of these were restricted to areas north of the United States-Mexican boundary. A recent Russian pharmacological investigation indicated that extracts of *T. minus* L. showed certain physiological effects on the heart when tested on frogs, cats, and dogs (2). An intravenous injection of the hydrochlorides of the extract of the total alkaloids exerted an effect on blood pressure and pulse. These discoveries provided the stimulus for this preliminary investigation of *T. dasycarpum* Fisch. & Lall. (Purplish Meadow Rue, Tall Meadow Rue).

In the early 1800's the name "pseudorhubarb" was commonly attributed to *T. flavum* L. as a result of its purgative properties. *T. flavum* was also thought to possess diuretic and febrifuge properties. In Southern Russia fomentations of the roots of *T. minus* were used as a family remedy against the bites of vipers (5).

M. E. Doassans (7, 8, 13) isolated an active principle from an extract of *T. macrocarpum* Gren. The principle was a colorless crystalline substance having pronounced toxic properties analogous to those of curare. These needle-shaped crystals were characterized as slightly soluble in water, soluble in alcohol, possessing the reactions of alkaloids, and capable of combining with acids to form soluble salts in water. To this substance he gave the name thalictrine. A second substance, yellow, soluble in water and devoid of any physiological properties, representing the coloring principle of *T. macrocarpum*, was subsequently isolated and given the name macrocarpine. Later Doassans and Mousset (9, 13) isolated berberine from *T. flavum* (Fen Rue, Monks Rhubarb). Rochebrune (9) reported the presence of thalictrine and macrocarpine in the roots of Spanish *T. glaucum* Desf. [*T. rugosum* Ait. (10)]. This work revealed that thalictrine was an active cardiac poison producing loss of power, convulsive movements, irregularity and depression of the heart beat, and finally death in some cases by convulsions. Rochebrune (9) also established the presence of thalictrine in African *T. rhyncocarpum* Dill & Rich.

Later chemical investigations of various species were concerned with cyanogenetic substances (11, 12). Klein (14) reported the presence of an unidentified alkaloid in the rhizomes of *T. aquilegifolium* L. Alkaloids were reported in plentiful amounts in *T. alpinum* L., (15) a wild-growing species of central Asia. *T. collinum* Wallr., *T. angustifolium* L., and *T. silvaticum* Koch. have been used in Ukrainian folk medicine for a variety of purposes, of which the major use appeared to be as a diuretic (16). Tests carried out in the phytochemical laboratory of the Ukrainian Scientific Research Institute for Chemistry and Pharmaceutics showed that *T. collinum* and *T. angustifolium* contained alkaloids (16). The rhizome of *T. foliolosum* DC. is considered to be a tonic and laxative and a

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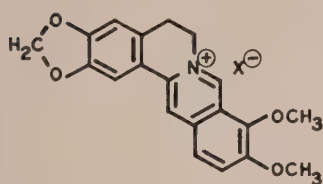
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good substitute for rhubarb, but it is mainly used in India and Afganistan as a substitute for mamira in the preparation of collyria in ophthalmia (17). Phytochemical investigation of the rhizomes of *T. foliolosum* by Vashistha and Siddiqui (17) revealed the presence of two alkaloids: berberine isolated as the iodide, and a quaternary alkaloid, thalictrine, as the iodide. A later investigation by Chatterjee and co-workers (18) of the rhizomes of *T. foliolosum* yielded berberine ($C_{20}H_{18}O_4NOH$) 0.35%, palmatine ($C_{21}H_{22}O_4NOH$) 0.03%, jatrorrhizine ($C_{20}H_{20}O_4NOH$) 0.02%, but thalictrine was absent. They suggested that thalictrine was possibly a mixture of palmatine and jatrorrhizine. However, Gopinath *et al.* (19) reported the isolation of thalictrine from the rhizomes of *T. foliolosum* and proved it to be identical with magnoflorine by mixed melting point determination of the iodide (mp 258° C d.) and of the picrate (mp 206–207° C d.) and by comparison of infrared spectra. Nakajima (20) reported the presence of two ether soluble alkaloids in *T. minus* var. *elatum* used as a home remedy in Japan. The chief alkaloid, elatine ($C_{40}H_{56}O_6N_3$), crystallized as colorless needles. The other alkaloid, a phenolic base, was unidentified. *T. simplex* L. was examined by Norkina and Pakhareva, (21) and alkaloids were found largely in the leaves and roots. The leaves yielded thalictrine ($C_{38}H_{46}N_2O_7$), mp 170° C, $[\alpha]_D^{25} - 80.9^\circ$ ($CHCl_3$). Yunusov and Progressov (22) isolated four alkaloids from the roots of *T. minus*. They reported (23) four alkaloids: thalmine ($C_{20}H_{23}O_3N$), mp 253° C; thalmine ($C_{21}H_{25}O_4N$), mp 192–193° C d.; thalmine ($C_{21}H_{25}O_5N$), mp 137–138° C, $[\alpha]_D + 255.3^\circ$; and thalmine ($C_{20}H_{25}O_4N$), mp 192–193° C, $[\alpha]_D - 84^\circ$. These appear to be oxygenated aporphine alkaloids. Burger (24) states that the properties of thalmine agree with those of glaucine, and undoubtedly thalmine is O-desmethylglaucine; however, the structure for thalmine may be incorrect due to some false assumptions in the elucidation. Fujita and Tomimatsu (25) carried out chemical studies of *T. thunbergii* DC. From the roots they isolated magnoflorine ($C_{20}H_{24}O_4NOH$), mp 252° C d. (iodide), $[\alpha]_D^{12} + 214^\circ$, the first isolation of this quaternary alkaloid from the Ranunculaceae. Subsequently magnoflorine was isolated from the leaves (26). The leaves yielded a second quaternary alkaloid, takatonine (27), (1-(4-methoxybenzyl)-2-methyl-6,7,8-trimethoxyisoquinoline. Three tertiary bases (27–29) were also isolated from *T. thunbergii*: thalchuberine ($C_{21}H_{23}O_4N$), mp 126–127° C, 1-(2-dimethylaminoethyl)-3,4-dimethoxy-6,7-methylene-dioxy phenanthrene from the roots; thalchuberine ($C_{37}H_{40}O_6N_2$), mp 161° C; and O-methylthalchuberine ($C_{38}H_{42}O_6N_2$), mp 186–187° C d., from the stems and leaves. *T. isopyroides*, a plant growing in central Asia, was examined and found to contain alkaloids (30). Partial elucidation of one of these has given the empirical formula $[C_{19}H_{14}O(NCH_3)(OCH_3)_2(CH_2O_2)]$.

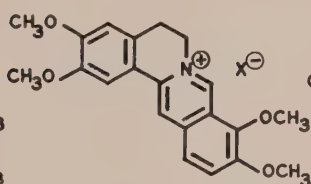
Thus, sixteen species of *Thalictrum* have been reported to contain alkaloids and are listed in table 1. Fifteen different alkaloids have been isolated and identified. The structures of some of these alkaloids are presented in figure 1. A total of twenty-two alkaloids have been isolated from nine species and one variety. Seven species studied have been determined to contain alkaloids but these have not been isolated and characterized.

EXPERIMENTAL

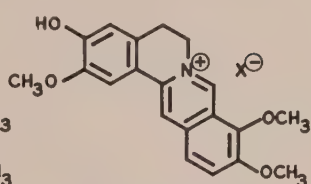
Procurement of Plant Material and Extraction of Roots.—Whole plants of *Thalictrum dasycarpum* Fisch. and Lall., var. *hypoglaucum* (Rydb.) Boivin which grow in prairies and open woodlands of eastern Kansas were collected in June, 1959, in the vicinity of Lawrence, Kansas. The plants were air dried and the tops were separated from the roots. The root was passed through a Wiley mill. The extraction procedure utilized was a modification of a method reported by Manske (33). The milled root (940 g) was extracted with 95 per percent ethanol (1200 ml) utilizing a continuous extractor. The extraction was carried to exhaustion as



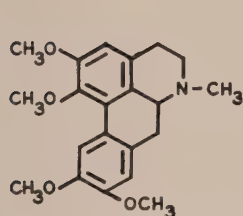
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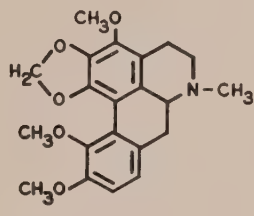
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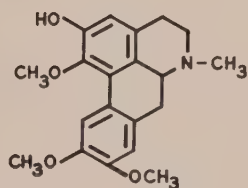
JATRRORRHIZINE



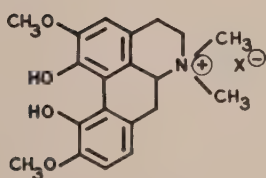
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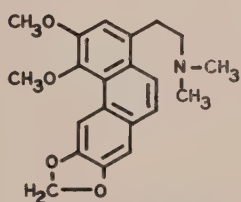
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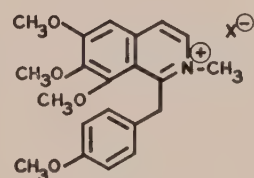
THALICMIDINE



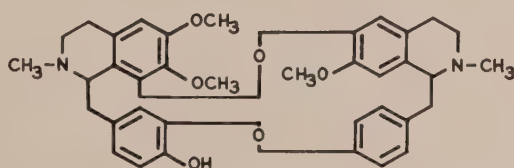
MAGNOFLORINE



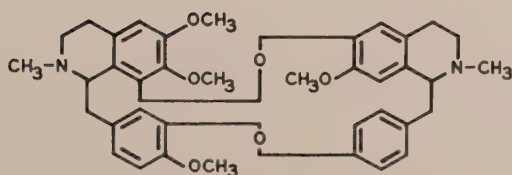
THALICTHUBERINE



TAKATONINE



THALICTBERINE



O-METHYLTHALICTBERINE

FIG. 1. Structures of some *Thalictrum* alkaloids.

indicated by a negative test with Valser's reagent (34). The extract was concentrated almost to dryness, poured slowly with constant stirring into a warm, aqueous hydrochloric acid solution (*pH* 2) placed in the refrigerator for 36 hours. Insoluble material separated leaving a clear supernatant solution which was carefully decanted. The insoluble material was repeatedly extracted with the acid solution until the extractive gave a negative test with Valser's reagent. The combined decantates were concentrated to approximately 400 ml and extracted to exhaustion, at *pH* 2, with methylene chloride utilizing a continuous liquid-liquid extractor. The methylene chloride extract was evaporated *in vacuo* to yield 19.06 g crude material and was designated "neutral fraction" (*NF*).

TABLE 1. Alkaloids isolated from *Thalictrum* species.

Species	Plant part ¹	Alkaloid	Formula	Melting point, C	Reference
<i>T. macrocarpum</i> Gren.	r.	Thalictrine	Unknown		7, 8, 9, 13
<i>T. rhynchocarpum</i> Dill and Rich.	r.	Thalictrine	Unknown		9
<i>T. glaucum</i> Desf. (<i>T. rugosum</i> Ait.)		Thalictrine	Unknown		9
<i>T. flavum</i> L.	r.	Berberine	$C_{20}H_{19}O_4NOH$	145°	13
<i>T. aquilegifolium</i> L.	rh.	Unknown			14
<i>T. alpinum</i> L.	w.	Unknown			15
<i>T. foliolosum</i> D C.		Thalictrine	$C_{20}H_{27}O_4N$	208° d	17, 18, 19
		(Magnoflorine)	$C_{20}H_{24}O_4NOH$	258° d (Iodide)	
	rh.	Berberine	$C_{20}H_{18}O_4NOH$	145°	17, 18
	rh.	Jatrorrhizine	$C_{20}H_{20}O_4NOH$	203-204° (Iodide)	18
		Palmatine	$C_{21}H_{22}O_4NOH$	241° (Iodide)	18
<i>T. minus</i> var. <i>elatum</i>	w.	Elatrine	$C_{27}H_{55}O_6N_3$		20
<i>T. simplex</i> L.	l.	Thalictrinine	$C_{38}H_{46}N_2O_7$	170°	21
<i>T. hernandezii</i> Tausch	r.	Unknown			31
<i>T. polygamum</i> Muhl.	fl, l, r	Unknown			32
<i>T. minus</i> L.	r.	Thalmine	$C_{20}H_{23}O_3N$	253°	22, 23
	r.	Thalmidine	$C_{21}H_{25}O_4N$	192-193° d.	22, 23
	r.	Thalicmine	$C_{21}H_{25}O_5N$	137-138°	22, 23, 24
	r.	Thalicmidine	$C_{20}H_{25}O_4N$	192-193°	22, 23, 24
	r.	d-Glaucine	$C_{21}H_{25}O_4N$	120°	24
<i>T. thunbergii</i> D C.	r.	Takatonine			27
	r.	Thalichthuberine	$C_{21}H_{23}O_4N$	126-127°	27
	l, r, s	Magnoflorine	$C_{20}H_{24}O_4NOH$	252° d (Iodide)	25, 26
	l, s	Thalicerberine	$C_{37}H_{40}O_6N_3$	161°	28
	l, s	O-Methyl Thalicerberine	$C_{38}H_{42}O_6N_2$	186-187° d.	28
<i>T. isopyroides</i>	l, r, s	Unknown			30
<i>T. collinum</i> Wallr.	l, r, s	Unknown			16
<i>T. angustifolium</i> L.	l, r, s	Unknown			16
<i>T. dasycarpum</i> Fisch. and Lall.	r.	Magnoflorine	$C_{20}H_{24}O_4NOH$		
var. <i>hypoglaucum</i> (Rydb.) Boivin.		Berberine	$C_{20}H_{18}O_4NOH$		

¹fl, flowers; l, leaves; r, roots; rh, rhizome; s, seeds; w, plant.

The aqueous solution was basified with ammonium hydroxide to *pH* 8 and extracted with methylene chloride. The methylene chloride extract was evaporated *in vacuo* to yield 10.3 g brown material containing tertiary alkaloids and was designated "tertiary fraction" (*TF*).

The alkaline mother-liquor was adjusted to *pH* 4 with 10 per cent hydrochloric acid and the quaternary bases were precipitated with ammonium reineckate following a modified procedure of Panouse (35).

A saturated aqueous solution of reineckate salt (2 g/100 ml) was poured slowly with constant stirring into the aqueous acidic (*pH* 4) extract until precipitation ceased. The solution was allowed to stand in the refrigerator for 24 hours. The precipitate, collected on a Buchner funnel, was dried under suction. The precipitate was washed with ether to remove extraneous matter and to facilitate drying.

The precipitate was treated with acetone (800 ml) until all acetone soluble material was removed. The solution was concentrated and an equal volume of water (300 ml) was added. The filtrate alkaloids were converted to the chloride after the method of Kapfhammer (36). Silver reineckate was precipitated by addition of 345.6 ml silver sulfate solution (6 g/1000 ml). The silver reineckate was removed by filtration and the filtrate was treated with 158.9 ml barium chloride solution (2.56 g $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ in 250 ml water) as previously calculated [1 ml Ag_2SO_4 (6g/1000 ml) = 0.46 ml $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ (10.24 g/1000 ml)] and filtered through celite to remove barium sulfate. The acetone was removed from the clear filtrate by evaporation *in vacuo* and the remaining aqueous solution was freeze-dried, yielding 6.61 g quaternary chlorides (QCl).

Paper Chromatography.—The descending paper chromatographic technique was employed using Whatman No. 1 paper. The solvent systems used were: ethyl acetate-pyridine-water (EPW), 750:310:165; butanol-ammonium hydroxide-water (BAW), 35:5:1; and propanol-ammonium hydroxide-water (PAW), 4:2:1. The papers were equilibrated over night previous to development. The chromatograms were examined under ultraviolet light (long wave) and sprayed with a modified Dragendorff's reagent (37). The chromatograms were washed, after spraying, with 1 per cent acetic acid solution permitting the orange spots to be observed more readily against a white background.

Ultraviolet, Infrared, Optical Rotation, and Melting Point Measurements.—A Beckman DU (Model 2400) spectrophotometer was used for the determination of the ultraviolet spectra. Where acid spectra are specified, one ml of 0.1N hydrochloric acid in 95 per cent ethanol was diluted to 10 ml with 95 per cent ethanol solution of the sample to be measured. Where basic spectra are specified, one ml of 0.1N ammonium hydroxide in 95 per cent ethanol was diluted to 10 ml with 95 per cent ethanol solution of the sample to be measured. The infrared spectra were obtained with a Perkin-Elmer recording spectrophotometer (Infracord) as potassium bromide pellets. Optical rotation values were taken in a 2 dm glass polarimeter tube using methanol as the solvent. The melting points are corrected and were taken with a Fisher-Johns melting-point apparatus.

NEUTRAL ALKALOID FRACTION

Berberine Iodide.—The NA fraction (19.06 g) was extracted with 300 ml petroleum ether (bp 30–60° C). The petroleum ether insoluble residue was dissolved in 525 ml distilled water and all but a small amount of black material dissolved. The aqueous solution was filtered and the filtrate adjusted to pH 8 with ammonium hydroxide. The brownish-black precipitate which formed was collected (a) and the filtrate (b) was reserved. The precipitate (a) was dissolved in diluted hydrochloric acid and filtered. The filtrate (c) was adjusted to pH 8 and the precipitate (d) which formed was collected and reserved for future investigation. The filtrate (e) was combined with filtrate (b) above. The combined filtrates were adjusted to pH 8 with ammonium hydroxide and extracted with ether (300 ml), and were reserved for future investigation. The alkaline aqueous solution was made acidic (pH 4) with hydrochloric acid and treated with ammonium reineckate as previously described. The precipitated reineckate (f) was collected, semi-dried under suction, and washed with ether. The reineckate was dissolved in acetone (200 ml) and an equal amount of water was added. The precipitate (g) was collected, dissolved in acetone, and water added just short of precipitation. The reineckate filtrate (h) of (g) and the solution of (g) were decomposed and converted to the chloride as described previously. Lyophilization of the chlorides yielded 0.59 g homogenous material (from g) and 1.10 g other alkaloids (from h). The latter material was reserved for future investigation. The lyophilized chloride (from g) was dissolved in hot methanol (30 ml) to which a small amount of saturated potassium iodide solution was added. The resulting solution was allowed to stand

over night whereupon it crystallized. The crystals were filtered, washed with a small amount of methanol, and recrystallized from 15 ml methanol, yielding 420 mg yellow crystalline iodide (I).

Superimposed spotting of (I) and authentic berberine iodide revealed the presence of a single yellow fluorescent spot under ultraviolet light which stained with Dragendorff's reagent when chromatographed with three solvent systems. The R_F values were as follows: PAW, 0.43; BAW, 0.41; EPW, 0.35. Compound (I) and berberine iodide gave identical ultraviolet spectra (table 2) and superimposable infrared spectra.

Berberine Chloride.—The iodide salt (I) was converted to the chloride by an ion-exchange resin. Twenty-five ml of Amberlite IRA-410 was placed in a glass column (1 cm in diameter) and washed with 0.05N hydrochloric acid and distilled

TABLE 2. Ultraviolet spectra

Compound	$\lambda_{\max.}$ (log E)					
	Neutral		Acid		Base	
Berberine iodide I.	227 μ	(4.68)	227 μ	(4.70)	227 μ	(4.68)
	268	(4.57)	268	(4.60)	268	(4.57)
	348	(4.54)	348	(4.56)	348	(4.53)
Berberine chloride II.	231 μ	(4.43)	231 μ	(4.44)	231 μ	(4.27)
	267	(4.44)	267	(4.44)	267	(4.36)
	349	(4.41)	349	(4.40)	349	(4.36)
13,14-Dihydro-9-desoxy-berberine (III).....	288 μ	(3.76)	288 μ	(3.81)	288 μ	(3.73)
Magnoflorine perchlorate (IV).....	228 μ	(4.49)	224 μ	(4.56)	231 μ	(4.48)
	268	(4.07)	268	(4.14)	279	(4.15)
	303	(3.83)	303	(3.81)	328	(3.89)
Magnoflorine iodide (V)....	223 μ	(4.67)	222 μ	(4.90)	226 μ	(4.63)
	269	(3.97)	268	(4.17)	277	(3.81)
	311	(3.81)	303	(3.88)	328	(3.92)
O,O-Dimethyl magnoflorine iodide (VI).....	223 μ	(4.80)	223 μ	(4.71)	223 μ	(4.71)
	273	(4.20)	273	(4.22)	273	(4.22)
	299	(3.78)	299	(3.79)	299	(3.79)
O,O-Dimethyl magnoflorine chloride (VII).....	225 μ	(4.57)	225 μ	(4.65)	225 μ	(4.54)
	273	(4.10)	273	(4.10)	273	(4.08)
	299	(3.73)	299	(3.71)	299	(3.70)

water. The alkaloid iodide (I, 120 mg) was dissolved in 20 ml water to which was added enough methanol to effect solution and washed slowly through the column with water. Evaporation of the eluate gave the chloride salt (II), 110 mg.

Superimposed spotting of (II) and berberine chloride gave a single spot when chromatographed with two of the solvent systems. The spot fluoresced yellow under ultraviolet light and stained when sprayed with Dragendorff's reagent. The R_F values were as follows: PAW, 0.64; BAW, 0.20. Compound (II) and berberine chloride gave identical ultraviolet spectra (table 2) and superimposable infrared spectra.

13,14-Dihydro-8-desoxyberberine.—The iodide (I) was reduced following the method of Awe, Wichman and Buerhop (38). The alkaloid iodide (I, 101.5 mg) was dissolved in 70 ml methanol at 25° C and NaBH_4 was added until the solution was colorless. The mixture was allowed to stand for 30 minutes before evaporating

the solvent *in vacuo*. After drying in a vacuum desiccator the residue was extracted several times with methylene chloride ($8 \times 6 = 48$ ml). The methylene chloride solution was evaporated and the residue crystallized from methanol to yield 72 mg colorless crystals (III). The melting point ($169\text{--}170^\circ\text{C}$) was the same as an authentic sample of 13,14-dihydro-8-desoxyberberine and was undepressed by admixture with this sample. Awe, *et al.* (38) reported mp 172°C for this compound.

Compound III superimposed with 13,14-dihydro-8-desoxyberberine gave a single spot when chromatographed using two solvent systems. The spot fluoresced light yellow under ultraviolet light and stained with Dragendorff's reagent. The R_F values were as follows: PAW, 0.93; BAW, 0.91. Compound III and 13,14-dihydro-8-desoxyberberine gave identical ultraviolet spectra (table 2) and superimposable infrared spectra.

QUATERNARY ALKALOID FRACTION

Magnoflorine Perchlorate.—QCl was passed through a chromatographic column, 2 cm in diameter, of Woelm neutral alumina (120 g, activity grade IV) as a preliminary purification procedure. The fraction (6.61 g) was taken up in a small amount of methanol and mixed with 5 g alumina, dried, passed through a sieve to break up any lumps, and placed on top of the column. The chlorides were eluted with methanol. The eluate was evaporated to near dryness, and streaked on large sheets ($46\text{ cm} \times 57\text{ cm}$) of Whatman No. 16 paper. The mixture was chromatographed, after equilibrating over night, with PAW solvent system. The resulting paper chromatogram was observed to develop into the following pattern: (a) a large, blue fluorescent streak (under ultraviolet light) at the top, (b) a narrow, visible yellow streak which was nonfluorescent and which turned red in the presence of ammonium hydroxide, (c) a small, blue-green fluorescent streak, and (d) a narrow, dark brown band at the bottom. Streak (a) was cut out and the substance eluted with methanol which on evaporation yielded crystalline material. Streaks (b, c, and d) were treated similarly and the eluates reserved for future investigation. The crystalline material (900 mg) obtained from streak (a) was chromatographed and found to be composed of two blue fluorescent materials with nearly identical R_F values (PAW, 0.39 and 0.41) and both stained intensely with Dragendorff's reagent. The crystalline mixture in glacial acetic acid, was heated on a steam bath and the material which did not dissolve was filtered (50 mg). The filtrate was reduced to a total volume of 50 ml *in vacuo*. Perchloric acid (70 per cent, 4 drops) was added to the solution at room temperature. The solution was allowed to stand over night and the precipitate (653 mg) was recrystallized from glacial acetic acid to give a light tan material. Paper chromatography indicated that the crystalline perchlorate was a mixture of two blue fluorescent compounds. It was found that the two compounds could be separated by virtue of the fact that the main component was soluble in acetone and the other compound was insoluble in acetone. The crystalline perchlorate mixture was repeatedly washed with acetone until nearly all the soluble material had been obtained (480 mg). Paper chromatographic studies indicated one blue fluorescent compound to be present. This material (IV) gave a melting point of $257\text{--}258^\circ\text{C}$ d. and was undepressed when admixed with an authentic sample of magnoflorine perchlorate (39). Optical rotation: $[\alpha]_D^{33} + 218$ (7.8 mg/5 ml CH_3OH). Reported (39): mp $256\text{--}258^\circ\text{C}$ d.; $[\alpha]_D^{24} + 215^\circ$ (CH_3OH).

Superimposed spotting of Compound (IV) and magnoflorine perchlorate gave a single spot when chromatographed with two of the solvent systems. The spot fluoresced blue under ultraviolet light and stained with Dragendorff's reagent. The R_F values were as follows: PAW, 0.43 BAW, 0.41. Compound (IV) and magnoflorine perchlorate gave identical ultraviolet spectra (table 2) and superimposable infrared spectra.

Magnoflorine Iodide.—The perchlorate salt (IV, 369 mg) was converted to the chloride by using Amberlite IRA-410 resin as previously described. The chloride was converted to the iodide in the manner described under berberine iodide. A crystalline iodide was obtained (V, 352 mg). The melting point 249°C d. was undepressed when admixed with magnoflorine iodide. Optical rotation $[\alpha]_{\text{D}}^{27} + 209^{\circ}$ (7.2 mg/5 ml of CH_3OH). Reported (25): mp 252°C d., $[\alpha]_{\text{D}}^{12} + 214^{\circ}$ (CH_3OH).

Superimposed spotting of Compound (V) and magnoflorine iodide exhibited a single spot when chromatographed utilizing two solvent systems. The spot fluoresced blue and stained with Dragendorff's reagent. The R_F values were as follows: PAW, 0.375; BAW, 0.024. Compound (V) and magnoflorine iodide gave identical ultraviolet spectra (table 2) and superimposable infrared spectra.

O,O-Dimethylmagnoflorine Iodide.—Compound (V) was converted to O,O-dimethylmagnoflorine iodide by the method of Nakano (40). To a methanolic solution of compound (V, 121 mg.), a methanolic solution of 125 mg of potassium hydroxide and an excess of methyl iodide were added. The mixture was refluxed for six hours and a similar amount of potassium hydroxide and methyl iodide again were added. This was repeated four times. Subsequently a methanolic solution of 100 mg of potassium hydroxide and an excess of methyl iodide were added and the mixture refluxed for a further six hours. After repeating this process two times, the solution was evaporated *in vacuo* and the residue which contained a large amount of inorganic material was extracted with 300 ml chloroform. The chloroform solution was dried over anhydrous potassium carbonate, filtered and concentrated, depositing crystals. Recrystallization from methanol-acetone yielded 61 mg of colorless needles (VI), mp $243\text{--}244^{\circ}\text{C}$ d. Reported for O,O-dimethylmagnoflorine iodide: mp $243\text{--}244^{\circ}\text{C}$ d. (25); mp $242.5\text{--}243^{\circ}\text{C}$ d. (40).

Superimposed compound (VI) and O,O-dimethylmagnoflorine iodide exhibited a single spot detectable only with Dragendorff's reagent when chromatographed. The R_F values were as follows: BPW, 0.785; BAW, 0.43; PAW, 0.82. Compound (VI) and O,O-dimethylmagnoflorine iodide gave identical ultraviolet spectra (table 2) and superimposable infrared spectra.

O,O-Dimethylmagnoflorine Chloride.—A sample (10.6 mg) of (VI) was dissolved in methanol containing a small amount of water and shaken with freshly prepared silver chloride (from 150 mg silver nitrate). After one hour the solution, free of iodide ions, was filtered and evaporated to dryness *in vacuo*. The residue was crystallized from ethanol-acetone solution and yielded 7.2 mg colorless prisms, mp $237\text{--}238^{\circ}\text{C}$ d. (Compound VII). Nakano (40) obtained a melting point of $236\text{--}237^{\circ}\text{C}$ d. for O,O-dimethylmagnoflorine chloride.

When (VII) was superimposed with O,O-dimethylmagnoflorine chloride it gave a single nonfluorescent spot which stained with Dragendorff's reagent using PAW solvent system (R_F 0.906). The ultraviolet spectra of VII and O,O-dimethylmagnoflorine chloride were identical (table 2).

SUMMARY AND CONCLUSIONS

Alkaloids obtained from the roots of *Thalictrum dasycarpum* Fisch. and Lall., var. *hypoglaucum* (Rydb.) Boivin by ethanol extraction were divided into three fractions which were as follows: a "neutral fraction", a "tertiary fraction", and a "quaternary fraction".

From the "neutral fraction" was isolated an alkaloid identified as berberine. The identification was based upon paper chromatographic data, melting points, mixed melting points, ultraviolet spectra, and infrared spectra of the following derivatives: berberine iodide, berberine chloride, and 13,14-dihydro-8-desoxy-berberine.

From the "quaternary fraction" was isolated an alkaloid identified as magnoflorine. The identification was based upon the same type of data of the following

derivatives: magnoflorine perchlorate, magnoflorine iodide, O,O-dimethylmagnoflorine iodide, and O,O-dimethylmagnoflorine chloride.

The investigation of further alkaloidal constituents of *T. dasycarpum* and other *Thalictrum* species is being continued in our laboratories.

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